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(54) **Titre : APPAREIL ET PROCEDES POUR LA SYNTHESE AUTOMATISEE DE PETITES MOLECULES**
(54) **Title: APPARATUS AND METHODS FOR THE AUTOMATED SYNTHESIS OF SMALL MOLECULES**

(57) **Abrégé/Abstract:**
Provided are methods for purifying N-methyliminodiacetic acid (MID A) boronates from solution. Also provided are methods for deprotection of boronic acids from their MIDA ligands. The purification and deprotection methods can be used in conjunction with methods for coupling or otherwise reacting boronic acids. Iterative cycles of deprotection, coupling, and purification can be performed to synthesize chemical compounds of interest. The methods are suitable for use in an automated chemical synthesis process. Also provided is an automated small molecule synthesizer apparatus for performing automated synthesis of small molecules using iterative cycles of deprotection, coupling, and purification in accordance with methods of the invention. Coupling and other reactions embraced by the invention include, without limitation, Suzuki-Miyaura coupling, oxidation, Swern oxidation, "Jones reagents" oxidation, reduction, Evans' aldol reaction, HWE olefmation, Takai olefmation, alcohol silylation, desilylation, /?-methoxybenzylation, iodination, Negishi cross-coupling, Heck coupling, Miyaura borylation, Stille coupling, and Sonogashira coupling.

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Apparatus and Methods for the Automated Synthesis of Small Molecules

RELATED APPLICATIONS

This application claims the benefit of priority to United States Provisional Patent Application serial number 61/367,176, filed July 23, 2010.

GOVERNMENT SUPPORT

This invention was made with U.S. Government support under National Institutes of Health Grant Nos. GM080436 and GM090153. The government has certain rights in the invention.

BACKGROUND

Similar to peptides, oligonucleotides, and oligosaccharides, most small molecule natural products are highly modular in their constitution. This is because, like the aforementioned oligomers, the majority of small molecules are biosynthesized via the sequential coupling of bifunctional building blocks. Specifically, polyketides are derived from multiple malonyl-CoA and/or methmalonyl-CoA units, non-ribosomal peptides are built from amino acids, polyterpenes are stitched together from isopentenyl pyrophosphate and/or dimethylallyl pyrophosphate building blocks, and fatty acids are prepared from fragments of malonyl-CoA. Other classes of modular natural products result from the oxidative coupling of common building blocks, such as shikimic acid, amino acids, and/or their respective derivatives.

With peptides, oligonucleotides, and increasingly oligosaccharides, this inherent modularity is now routinely harnessed to enable fully automated syntheses from suitably protected bifunctional building blocks (R.B. Merrifield, *Science* **1965**, *150*, 178-185; M.H. Caruthers, *Science* **1985**, *24*, 799; and O.J. Plante, M.R. Palmacci, P.H. Seeberger, *Science* **2001**, *291*, 1523). As a direct result of these advances, research in theses areas is primarily focused on discovering and understanding new molecular function. In stark contrast, despite tremendous advances over the course of nearly two centuries, the laboratory synthesis of small molecules remains a relatively complex, inflexible, and non-systematized process practiced almost exclusively by highly-trained specialists. (For pioneering developments in the automated synthesis of small molecules via polymer-assistance and/or

flow chemistry, see: a) C.H. Hornung, M.R. Mackley, I.R. Baxendale and S.V. Ley and, *Org. Proc. Res. Dev.* **2007**, *11*, 399-405; b) Nikzad Nikbin, Mark Ladlow, and Steven V. Ley *Org. Process Res. Dev.* **2007**, *11*, 458–462; and c) France, S.; Bernstein, D.; Weatherwax, A.; Lectka, T. *Org. Lett.* **2005**, *7*, 3009-3012.) Thus, research in this area is still heavily weighted towards synthesis. Given the special properties of many small molecules that make them uniquely suited for a wide range of applications in science, engineering, and medicine, increased access to these compounds via a highly general and automated synthesis platform that is accessible to the non-expert would be highly enabling. Ultimately, such a process could help shift the primary focus from the synthesis of small molecules to the discovery and understanding of important small molecule functions.

SUMMARY

Certain aspects of the invention relate to an apparatus that utilizes a carbon-carbon bond-forming reaction iteratively to assemble a wide range of small molecules from pre-fabricated building blocks, and methods for using the same. In certain embodiments, analogous to the automated preparation of peptides from suitably protected amino acids, the automated process involves the controlled, iterative assembly of bifunctional haloboronic acid building blocks protected as the corresponding N-methyliminodiacetic acid (MIDA) boronates. In certain embodiments, obviating the need for any covalent attachment to a solid support, purification of intermediates is achieved by harnessing two remarkably general physical properties of MIDA boronates: the capacity for catch-and-release chromatography with silica gel; and their insolubility in hexanes. Additional aspects, embodiments, and advantages of the invention are discussed below in detail.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A depicts analogous strategies for the synthesis of peptides and small molecules.

Figure 1B is a schematic representation of exemplary iterated cycles of deprotection, cross-coupling, and purification with a final direct-release cross-coupling step, wherein for each coupling, roughly 3 equivalents of boronic acid are employed relative to each halide.

Figure 1C depicts two distinct strategies for purifying MIDA boronate intermediates. The first strategy is a "catch-and-release purification" and takes advantage

of the high affinity of MIDA boronates for silica gel. Specifically, a crude product mixture is loaded onto a pad of silica gel, which is then flushed with Et_2O/MeOH. While all byproducts are rapidly eluted in this polar solvent mixture, MIDA boronates show essentially infinite retention. Remarkably, the "catch" phenomenon is general for any compound that contains the MIDA boronate functional group. Simply switching the solvent to tetrahydrofuran (THF), however, "releases" the purified MIDA boronate as a solution suitable for use in the subsequent deprotection reaction. The second strategy is a "precipitation purification" and harnesses the general insolubility of MIDA boronates in hexanes. Specifically, a crude reaction mixture in THF is transferred into a chamber containing hexanes. The MIDA boronate precipitates, and is separated from the soluble reaction byproducts via filtration. A customized hybrid purification vessel may be used to harness both of these purification processes in series, thus providing a highly robust and general method for automated purification of MIDA boronate intermediates without the need for covalent attachment to a solid support.

Figure 1D depicts a photograph of one embodiment of a fully automated small molecule synthesizer comprising modules for (i) deprotection, (ii) cross-coupling, and (iii) purification, all of which are under the control of a computer equipped with custom-designed software.

Figure 2 depicts examples of reactions which may be run in a synthesizer, wherein D represents a deprotection step, CC represents a cross-coupling step, P represents a purification step, and RCC represents a cross-coupling step with either fast or slow *in situ* release of the boronic acid/boronic ester.

Figures 3 depicts selected building blocks, including bifunctional haloboronic acid building blocks protected as the corresponding N-methyliminodiacetic acid (MIDA) boronates, and compounds which can be prepared from said building blocks.

Figure 4 depicts selected building blocks, including bifunctional haloboronic acid building blocks protected as the corresponding N-methyliminodiacetic acid (MIDA) boronates, and compounds which can be prepared from said building blocks.

Figure 5 depicts (top) a design schematic of one embodiment of an automated small molecule synthesizer and (bottom) an example of the connectivity of the various pumps, valves, ports and tubes; wherein (1) denotes solvent reservoirs; (2) denotes a drying and degassing table; (3) denotes a heating block and stir-plate; (4) denotes solenoid valves and

gas manifolds; (5) denotes a deprotection table; (6) denotes a purification table; (7) denotes a valve module (with one example of a valve map shown in Figure 7); (8) denotes main syringe pumps; (9) denotes a syringe pump for purification; and (10) denotes a syringe pump for aqueous reactions.

Figure 6 depicts photographs of exemplary reaction tubes, tubing and fittings.

Figure 7 depicts an example of a valve map.

Figure 8 depicts an example of a reaction tube.

Figure 9 is a schematic of an example of an aqueous deprotection module.

Figure 10 depicts an example of a precipitation chamber and silica column.

Figure 11 depicts an example of a drying and degassing tube.

Figure 12 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 13 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 14 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 15 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 16 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 17 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 18 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the

mixture.

Figure 19 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 20 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 21 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 22 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 23 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 24 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 25 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 26 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 27 is a pair of ^{1}H NMR spectra corresponding to (i) a mock reaction mixture comprising a MIDA boronate, and (ii) the MIDA boronate after purification from the mixture.

Figure 28 depicts (**A**) automated aqueous deprotection of phenyl MIDA boronate and subsequent automated cross-coupling of phenyl boronic acid with a vinyl iodide bifunctional building block; (**B**), automated aqueous deprotection of trienyl MIDA boronate

and subsequent automated cross-coupling of trienyl boronic acid with a vinyl iodide bifunctional building block; and (**C**) automated aqueous deprotection of butenyl MIDA boronate and subsequent automated cross-coupling of butenyl boronic acid with an isomeric mixture of dienyl vinyl iodide bifunctional building blocks.

Figure 29 depicts fully automated synthesis of all-*trans*-retinal using an aqueous deprotection module.

DETAILED DESCRIPTION

Certain aspects of the present invention are directed to apparatuses and methods for the automated synthesis of small molecules. In certain embodiments, the small molecules are prepared by using a single reaction iteratively to unite a collection of bifunctional building blocks having all of the required functionality, oxidation states, and stereochemistry pre-installed.

BIFUNCTIONAL MIDA-PROTECTED HALOBORONIC ACIDS

A key to the development of apparatuses and methods for the automated synthesis of small molecules was the use of the Suzuki-Miyaura reaction to achieve the iterative cross-coupling (ICC) of bifunctional "haloboronic acids" (**Figure 1A**). However, in order to have an efficient automatable procedure, the development of a mild and selective method for reversibly-attenuating one end of each haloboronic acid was required to avoid random oligomerization. In this vein, the apparatuses and methods described herein take advantage of the finding that the trivalent ligand N-methyliminodiacetic acid (MIDA) can act as a switch to turn the reactivity of a boronic acid "off" and "on" under very mild conditions (E.P. Gillis, M.D. Burke *J. Am. Chem. Soc.* **2007**, *129*, 6716-6717; and U.S. Patent Application Publication No. 2009/0030238, which is hereby incorporated by reference in its entirety). This property of MIDA boronates has made it possible to prepare a variety of natural products via repeated cycles involving MIDA boronate deprotection, selective cross-coupling, and purification (**Figure 1B**; S.J. Lee, K.C. Gray, J.S. Paek, M.D. Burke *J. Am. Chem. Soc.* **2008**, *130*, 466-468; E.P. Gillis, M.D. Burke, *J. Am. Chem. Soc.* **2008**, *130*, 14084-14085; and E.M. Woerly, A.H. Cherney, E.K. Davis, M.D. Burke, *J. Am. Chem. Soc.* **2010**, *132*, 6941-6943). Further enabling their general utility as building blocks, MIDA boronates are uniformly air-stable, highly crystalline, monomeric, free-flowing solids that are fully compatible with a wide range of common synthetic reagents and silica

gel chromatography (E.P. Gillis, M.D. Burke *Aldrichimica Acta* **2009**, *42*, 17-27). In addition, more recent advances in methods for preparing MIDA boronate building blocks, and the discovery of their capacity for "slow-release" cross-coupling, have substantially expanded the utility of the synthesis platforms described herein (G.R. Dick, D.M. Knapp, E.P. Gillis, *Org. Lett.* **2010**, *12*, 2314-2317; D.M. Knapp, E.P. Gillis *J. Am. Chem. Soc.* **2009**, *131*, 6961-6963; and U.S. Patent Application Publication No. 2010/0121062, which is hereby incorporated by reference in its entirety). In fact, a rapidly growing collection of MIDA boronates, representing many of the substructures that commonly appear in natural products and pharmaceuticals, is now commercially-available. The expanding scope of the Suzuki-Miyaura reaction, which increasingly includes Csp^3-Csp^3 type couplings (M.R. Netherton, G.C. Fu, *Adv. Syn. Cat.* **2004**, *346*, 1525-1532), indicates that the potential generality of this ICC strategy is substantial.

PURIFICATION OF MIDA-PROTECTED ORGANOBORONIC ACIDS

Transforming an ICC approach into a fully automated process requires a general strategy for purifying the synthetic intermediates. In the case of peptides, oligonucleotides, and oligosaccharides this problem has been solved by linking the growing oligomer to a solid-support. At the end of each coupling reaction, the desired product is separated from residual solvents, reagents, and byproducts via a simple filtration. Albeit highly effective in these contexts, there are two major limitations of this purification approach as a foundation for ICC-based small molecule synthesis.

First, this strategy requires a ubiquitous chemical handle that enables covalent linking of the growing oligomer to the solid-phase. In the case of peptides, oligonucleotides, and oligosaccharides, the respective monomers all conveniently contain a common heteroatom linking element as an inherent component of the targeted structure. In contrast, although some excellent solid-phase linking systems have been developed, small molecules are quite structurally diverse, and many lack a common functional group available for attachment to a solid-phase.

Second, selectively coupling boronic acids in the presence of MIDA boronates requires that relatively anhydrous conditions be utilized because MIDA boronates are stable and unreactive under anhydrous basic conditions, but are readily hydrolyzed to yield reactive boronic acids when treated with aqueous base. In preliminary studies, it was found

that translating the chemistry of anhydrous Suzuki-Miyaura cross-couplings to the solid-phase can be problematic.

Surprisingly, the inventors have discovered two remarkable physical properties of MIDA boronates, allowing the circumvention of both of the aforementioned challenges. Collectively, the two properties have enabled a highly effective alternative purification strategy and, thus, allowed the complete automation of ICC with solution-phase chemistry. The two purification strategies -- "precipitation" and "catch-and-release" -- are discussed in detail below. The two purification strategies can be used alone or in combination, in which case they may be performed sequentially in either order.

Purification by Precipitation. One aspect of the invention relates to the discovery that virtually all molecules containing a MIDA-protected organoboronic acid functional group are insoluble in hexanes:THF (3:1 v/v), while almost all boronic acids, other boronic esters, or related surrogates are soluble in this solvent system (**Figure 1C**). This general physical property of MIDA boronates enables a highly efficient precipitation-based purification. (For background on precipitation-based purification see: H. Perrier, M. Labelle, *J. Org. Chem.* **1999**, *64*, 2110-2113; T. Bosanac, C.S. Wilcox, *Org. Lett.* **2004**, *6*, 2321-2324; and J.C. Poupon, A.A. Boezio, A.B. Charette, *Angew. Chem. Int. Ed.* **2006**, *45*, 1415-1420). Further, because most catalyst species and organic halides are soluble in hexanes:THF (3:1), MIDA boronates can be directly purified from cross-coupling reactions, such as anhydrous Suzuki cross-coupling reactions. Merely transferring a crude product mixture in THF (e.g., from a cross-coupling reaction) to a stirred vessel containing an amount of hexanes which is approximately three times the total volume of THF to be added results in rapid and quantitative precipitation of the MIDA boronate product while the residual unreacted boronic acid, as well as most byproducts and other reaction components, such as palladium and phosphine ligands, all remain soluble in the hexane:THF (3:1 v/v) mixture. Simple filtration of this mixture, followed by dissolution of the precipitated MIDA boronate with THF yields, a solution of semi-purified MIDA boronate.

One aspect of the invention relates to a method of purifying a MIDA boronate from a solution, comprising the steps of diluting with hexane the solution comprising the MIDA boronate, thereby selectively precipitating the MIDA boronate; and isolating the precipitated MIDA boronate. The hexane can be any isomer of hexane or a mixture of hexanes. Exemplary isomers of hexane useful in the invention include unbranched hexane (*n*-hexane), branched hexanes (e.g., isohexane), and cyclohexane.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the precipitated MIDA boronate is isolated by filtration.

In certain embodiments, the present invention relates to any one of the aforementioned methods, further comprising the step of dissolving the precipitated MIDA boronate in a polar solvent. In certain embodiments, the present invention relates to any one of the aforementioned methods, further comprising the step of dissolving the precipitated MIDA boronate in THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solution comprising the MIDA boronate is a THF solution.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solution comprising the MIDA boronate is added dropwise to the hexane.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the volume of hexane is between about two and about four times the volume of the solution comprising the MIDA boronate.

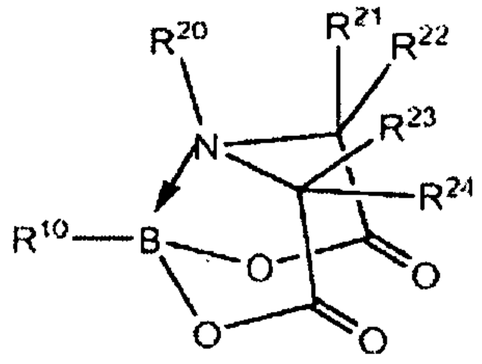
In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the volume of hexane is about three times the volume of the solution comprising the MIDA boronate.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solution comprising the MIDA boronate is a crude product mixture from a chemical reaction.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction comprises the step of contacting a

MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

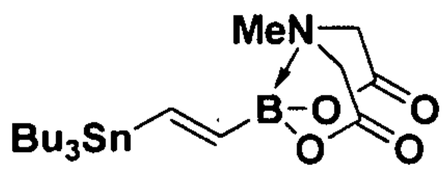
In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the MIDA boronate is represented by  ; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of a hydrogen group and an organic group.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

Catch-and-Release Purification. It has further been found that all molecules which contain a MIDA boronate functional group have exceptionally high affinity for silica gel (**Figure 1C**). For example, it has been discovered that MIDA boronates, regardless of the nature of the organic group appended to boron, have an R_f of essentially zero in hexanes:THF (3:1 v/v), Et_2O, and Et_2O:MeOH (98.5:1.5 v/v). Therefore, MIDA boronates can be used as a universal tag for catch-and-release purification on silica gel. (For an excellent review on tagging strategies for separations in organic synthesis, see: J. Yoshida, K. Itami, *Chem. Rev.* **2002**, *102*, 3693-3716. For an excellent review on modern separation techniques in organic synthesis, see: C.C. Tzschucke, C. Markert, W. Bannwarth, S. Roller, A. Hebel, R. Haag, *Angew. Chem. Int. Ed.* **2002**, *41*, 3964-4000. See, also, D.P. Curran, *Angew. Chem. Int. Ed.* **1998**, *37*, 1174-1196; P.H. Toy, K.D. Janda, *Acc. Chem. Res.* **2000**, *33*, 546-554; S.V. Ley, A. Massi, F. Rodriguez, D.C. Horwell, R.A. Lewthwaite, M.C. Pritchard, A.M. Reid, *Angew. Chem. Int. Ed.* **2001**, *40*, 1053-1055; A.R. Brown, S.L. Irving, R. Ramage, G. Raphy *Tetrahedron* **1995**, *51*, 11815-11830; L. A. Thompson, *Curr. Opin. Chem. Bio.* **2000**, *4*, 324-337; and M.G. Siegel, P.J. Hahn, B.A. Dressman, J.E. Fritz, J.R. Grunwell, S.W. Kaldor, *Tetrahedron Lett.* **1997**, *38*, 3357-3360. For the use of catch-and-release type methods to purify proteins, see: J. Porath, J. Carlsson, I. Olsson, G. Belfrage, *Nature* **1975**, *278*, 598.) In other words, the MIDA boronate functional group,

which is conveniently present in all intermediates utilized in certain ICC sequences (**Figure 1B**), enables the reversible non-covalent attachment of any MIDA boronate to silica gel, a solid support.

The use of hexanes:THF (e.g., 3:1 v/v) as a solvent system is important since it provides a means (via diluting with hexanes) to purify directly THF reaction solutions containing MIDA boronates. With regards to automated synthesis, as discussed below, this feature is important because advanced manipulations, such as solvent evaporation, are not required to prepare the reaction solution for purification. The use of Et_2O is important because in certain coupling reactions almost every other compound present in the reaction solution elutes in Et_2O. Interestingly, the addition of 1.5% MeOH (v/v) to the Et_2O ensures that even polar boronic acids are eluted off of the column with a reasonable amount of solvent. The compatibility of MeOH with MIDA boronates in the purification method was unexpected since MeOH can be used to deprotect MIDA boronates to the corresponding boronic acid at room temperature. All of the above-mentioned properties have been tested with many MIDA boronates and have been shown to be general. For example, boronates **A** and **B** behave as described above, despite the fact that they are non-polar and elute well in other solvent combinations, such as hexanes:EtOAc.



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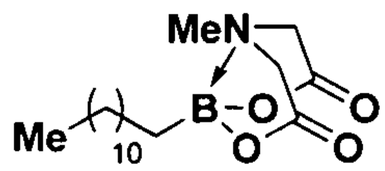


B

Once the unreacted boronic acids, as well as reaction regents, have been eluted, pure MIDA boronates generally elute well in THF. Also, MIDA boronates generally elute well with MeCN and acetone.

One aspect of the invention relates to a method of purifying a MIDA boronate from a solution, comprising the steps of passing the solution through a silica plug; passing a first liquid through the silica plug; and passing a second liquid through the silica plug, thereby eluting the MIDA boronate in the second liquid; wherein the first liquid contains diethyl ether or the polarity of the first liquid is less than or equal to about the polarity of a mixture of 98.5:1.5 (v/v) Et_2O:MeOH; and the polarity of the second liquid is greater than or equal to about the polarity of THF.

MIDA boronates, like most organic compounds, generally elute more rapidly off of SiO_2 (i.e., have a higher R_f) when the polarity of the solvent is higher. However, the

purification method described above takes advantage of special properties of MIDA boronates in certain solvents. Specifically, there are certain solvent systems in which the R_f of a MIDA boronate is not related to the polarity of the solvent. In fact, in certain solvent systems the R_f can approach or be zero. For example, even though chloroform is more polar than THF, the R_f of dodecyl MIDA boronate in chloroform is 0.00 and in THF is 0.80. While not intending to be bound by any particular theory, this very surprising phenomenon likely involves a unique interaction between all three factors: the solvent, silica gel and MIDA boronate. Thus, it is possible to isolate a MIDA boronate on a silica column if one picks a solvent that is an exception to the elution rules (such as chloroform or Et_2O). To remove a MIDA boronate from the column thus loaded, one switches to a polar solvent that obeys the normal elution rules (such as THF, MeCN, or acetone).

It has also been found that a functionalized silica gel, such as 3-aminopropyl-functionalized silica gel, can be substituted for SiO_2 without affecting the properties of the MIDA boronate/SiO_2 interaction. The functionalized silica gel can be used to scavenge, for example, metal catalysts from the solution. Therefore, in certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the silica is 3-aminopropyl-functionalized silica.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid comprises diethyl ether.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid is diethyl ether.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid is a mixture of diethyl ether and methanol.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein first liquid is a mixture of diethyl ether and methanol; and the ratio of diethyl ether to methanol is 98.5:1.5 (v/v).

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF, MeCN, ethyl acetate or acetone, or a solvent of similar polarity.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF, MeCN, ethyl acetate or acetone.

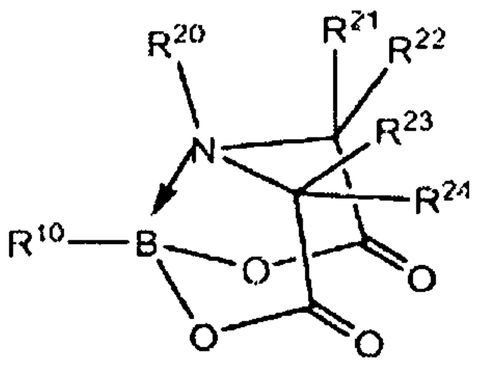
In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solution is a crude product mixture from a chemical reaction.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction comprises the steps of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the MIDA boronate is represented

by 

; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of a hydrogen group and an organic group.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

Combination Precipitation & Catch-and-Release Purification. The two purification strategies discussed above can be combined into one robust and general process. Specifically, the solution which is subjected to the catch-and-release purification described above can be a solution which is derived from the selective precipitation of a MIDA boronate.

One aspect of the invention relates to a method of purifying a MIDA boronate from a solution, comprising the steps of diluting the solution with hexane, thereby selectively precipitating the MIDA boronate; passing the diluted solution through a silica plug, thereby depositing the precipitated MIDA-protected organoboronic acid on the silica plug; passing a first liquid through the silica plug; and passing a second liquid through the silica plug, thereby eluting the MIDA boronate in the second liquid; wherein the first liquid contains diethyl ether or the polarity of the first liquid is less than or equal to about the polarity of a mixture of 98.5:1.5 (v/v) Et_2O:MeOH; and the polarity of the second liquid is greater than or equal to about the polarity of THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid comprises diethyl ether.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid is diethyl ether.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the first liquid is a mixture of diethyl ether and methanol.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein first liquid is a mixture of diethyl ether and methanol; and the ratio of diethyl ether to methanol is 98.5:1.5 (v/v).

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF, MeCN, ethyl acetate or acetone, or a solvent of similar or greater polarity.

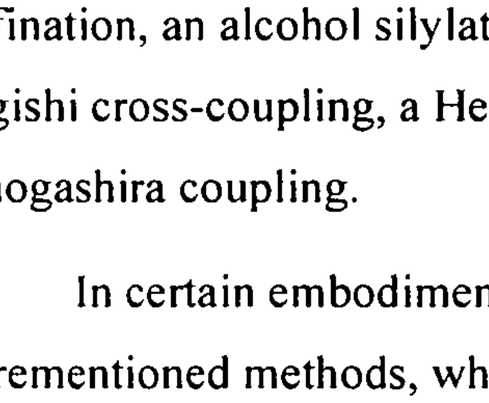
In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF, MeCN, ethyl acetate or acetone.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the second liquid is THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solution is a crude product mixture from a chemical reaction.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the chemical reaction comprises the steps of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the MIDA boronate is represented by  ; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of a hydrogen group and an organic group.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

Customized Hybrid Purification Vessels. In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein a customized hybrid purification vessel which contains both a "precipitation chamber" and a "catch-and-release chamber" arranged in series (**Figure 1C**) is used. In this system, a crude cross-coupling reaction is transferred to a first (e.g., upper) chamber filled with hexanes, resulting in rapid

and quantitative precipitation of the MIDA boronate-containing product while the residual boronic acid (and most byproducts), palladium, and phosphine ligand all remain soluble. As previously noted, simple filtration of this suspension, followed by washing with Et_2O:MeOH, places the resulting semi-purified, solid MIDA boronate on top of a silica gel plug that resides in a second (e.g., lower) chamber. This lower chamber is then subjected to washing with copious volumes of, for example, Et_2O:MeOH 98.5:1.5 (v/v) followed by a defined small volume of THF to effect the catch-and-release silica gel purification. The resulting THF solution of purified MIDA boronate is conveniently ready for utilization in subsequent cycles of deprotection and coupling.

PURIFICATION/DEPROTECTION OF MIDA-PROTECTED ORGANOBORONIC ACIDS

The challenges associated with purifying boronic acids include the fact that "the polar and often amphiphilic character tends to make their isolation and purification difficult" (Hall, D. G. *Boronic Acids*; Wiley-VCH: Weinheim, Germany, 2005; pp 57-58). Further, "[t]he widely known and used boronic acids show variable stability (vinyl-, alkyl-, and alkynylboronic acid are not very stable), and their purification is not straightforward. Moreover, isolated boronic acids generally contain large quantities of anhydrides or boroxines, which result in problems for determining their stoichiometry" (Darses, S.; Genet, J-P. *Chem. Rev.* **2008**, *108*, 288-325).

A number of approaches for purifying boronic acids have been developed, but all are limited in their generality. The most basic approach is to recrystallize the boronic acid, typically from an aqueous solution. However, this approach is only efficient if the sample is already relatively pure and when the temperature-dependent solubility of the boronic acid in water is favorable. When non-polar recrystallization solvents are employed, significant dehydration of the boronic acid to afford the boroxine can occur. (Santucci, L.; Gilman, H. *J. Am. Chem. Soc.* **1958**, *80*, 193-196). Another approach is "phase switching" liquid/liquid partitioning (Mothana, S.; Grassot, J-M.; Hall, D. G. *Angew. Chem. Int. Ed.* **2010**, *49*, 2883-2887). In this approach the boronic acid is converted into the anionic borate species in strong base (pH 10), non-anionic organics are washed away, and then the solution is acidified (pH 1-5) to regenerate the boronic acid. This method is not compatible with boronic acids containing acidic functional groups, basic functional groups, or any functionality that is acid-or base-sensitive, including the boronic acid functionality. A solid supported scavenger for boronic acids based on diethanolamine, abbreviated DEAM-PS,

has also been reported (Hall, D. G.; Tailor, J.; Gravel, M. *Angew. Chem. Int. Ed.* **1999**, *38*, 3064-3067). However, this method is expensive and does not represent a practical or scalable solution.

Boronic acids can be purified in a two-step process with the intermediacy of a boronic acid surrogate. For example, boronic acids can be converted to the corresponding trifluoroborate salt which can be crystallized (Darses, S.; Genet, J-P. *Chem. Rev.* **2008**, *108*, 288-325). However, limitations of this approach include the fact that the crystallization conditions are substrate-specific, large amounts of fluoride are used, some impurities co-crystallize with the product, and regenerating the boronic acid from the trifluoroborate is not efficient (Molander, G. A.; Cavalcanti, L. N.; Canturk, B.; Pan, P-S.; Kennedy, L. E. *J. Org. Chem.* **2009**, *74*, 7364-7369). Alternatively, boronic acids can be dehydrated in the presence of a diol (most often pinacol) to form the corresponding boronic ester. Some aryl boronic esters have more favorable chromatography, extraction, and crystallization properties than the corresponding boronic acids. However other classes of boronic esters (heteroaryl, alkenyl, alkyl, alkynyl, etc.) tend to have highly variable features. Further, as the boronic ester becomes stable enough to improve its purification properties, the conditions required to regenerate the boronic acid become harsher. For example, converting a pinacol boronic ester to the corresponding boronic acid typically requires aqueous acid and an oxidant (often $NaIO_4$), which limits the generality of this approach (Murphy, J. M.; Tzschuck, C. C.; Hartwig, J. F. *Org. Lett.* **2007**, *9*, 757-760).

Finally, unstable boronic acids present a particularly challenging problem. None of the above-mentioned approaches can be used to purify unstable boronic acids, such as vinyl boronic acids. Remarkably, vinyl boronic acid can be generated from vinyl MIDA boronate in greater than 95% purity (Knapp, D. M.; Gillis, E. P.; Burke, M. D. *J. Am. Chem. Soc.* **2009**, *131*, 6961-6963).

To address some of the problems noted above, disclosed herein is a "catch and selective release" type method developed for MIDA boronate hydrolysis. Specifically, a THF solution of a MIDA boronate (reactivity = OFF) is mixed with solid-supported ammonium hydroxide reagent (such as Amberlyst A26(OH); see T.M. Morwick, *J. Comb. Chem.* **2006**, *8*, 649-651) to promote the MIDA hydrolysis. At this point, both the cleaved MIDA ligand (likely in the form of $MIDA^{2-}Na^{+}_{2}$) and the boronic acid (likely in the form of the corresponding anionic boron-'ate' complex; see D.G. Hall, J. Tailor, M. Gravel, *Angew. Chem. Int. Ed.* **1999**, *38*, 3064-3067) remain trapped in the resin (the "catch"). It was

determined that subsequent treatment with a THF solution of AcOH (see M.G. Siegel, P.J. Hahn, B.A. Dressman, J.E. Fritz, J.R. Grunwell, S.W. Kaldor, *Tetrahedron Lett.* **1997**, *38*, 3357-3360) results in "selective release" of only the boronic acid (reactivity = ON), while the cleaved MIDA ligand conveniently remains trapped in the resin under these mildly acidic conditions. Transferring this THF/AcOH/boronic acid solution to a new vial containing K_2CO_3, 4 Å molecular sieves, and Celite®, followed by bubbling argon through the mixture and filtration yields a neutralized, mostly anhydrous, and deoxygenated solution of freshly-prepared boronic acid in THF, ready for the next cross-coupling reaction.

One aspect of the invention relates to the deprotection of a MIDA boronate, comprising the step of contacting a solution comprising the MIDA boronate and a solvent with a solid-supported ammonium hydroxide reagent, thereby deprotecting the MIDA boronate and forming a boronic acid and a MIDA.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solvent comprises THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent binds the MIDA.

In certain embodiments, the present invention relates to any one of the aforementioned methods, further comprising the steps of removing the solvent by filtration, thereby leaving the boronic acid and MIDA ligand trapped inside the solid-supported ammonium hydroxide reagent; and adding additional solvent.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the additional solvent is THF.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is washed with an organic solution comprising an organic solvent and a mild or strong acid in a quantity greater than that needed to neutralize the solid-supported ammonium hydroxide reagent, thereby eluting the boronic acid.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is

washed with a THF solution comprising a mild or strong acid, thereby eluting the boronic acid.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is washed with a THF solution comprising acetic acid, thereby eluting the boronic acid.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the eluted boronic acid is treated with base to neutralize the acid (e.g., acetic acid).

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the base is potassium carbonate.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is washed with a 1,4-dioxane solution comprising hydrochloric acid, thereby eluting the boronic acid.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is a strong base anion exchange resin , e.g., Amberlite IRA-400 (OH^- form), Amberlite IRA 420 (OH^- form), Amberlite IRA 410 (OH^- form), Amberlite IRN-150, Amberlite IRA 900 (OH^- form), Amberlite IRA 904 (OH^- form), Amberlite IRA 910 (OH^- form), Amberlite A5836, Amberlyst A26 (OH^- form), Ambersep 900, Dowex-1 (OH^- form), Dowex-3 (OH^- form), Dowex 1-X4 (OH^- form), Dowex 1-I 9880, Dowex 1-I0131, Dowex 550 A (OH^- form), or Amberjet 4400.

In certain embodiments, the present invention relates to any one of the aforementioned methods, wherein the solid-supported ammonium hydroxide reagent is a strong base, type 1, anionic, macroreticular polymeric resin based on crosslinked styrene divinylbenzene copolymer containing quaternary ammonium groups, e.g., Amberlyst A26 (OH^- form) (Rohm and Haas, Philadelphia, PA).

An aspect of the invention relates to a method of deprotecting a MIDA boronate, comprising the step of contacting a solution comprising the MIDA boronate and a solvent with an aqueous solution of NaOH, thereby deprotecting the MIDA boronate and forming a boronic acid and free MIDA ligand. This method is particularly useful in connection with

acid-sensitive substrates (boronic acids) because it does not include exposure to acid for elution from a solid support.

Because water will be removed in subsequent steps, it is generally desirable to limit the volume of the aqueous component (solution of NaOH) introduced into the system to a relatively small amount, e.g., about 25-33 percent of the volume of the solution comprising the MIDA boronate and its solvent.

In certain embodiments, the present invention relates to the aforementioned method, wherein the solvent comprises THF.

In certain embodiments, the present invention relates to the aforementioned method, wherein the solvent is THF. In one embodiment, the THF is dry and deoxygenated.

In certain embodiments, the present invention relates to the aforementioned method, further comprising the steps of adding diethyl ether, thereby generating a biphasic mixture comprising an organic phase comprising the boronic acid and free MIDA ligand, and an aqueous phase; and separating the organic phase comprising the boronic acid and free MIDA ligand from the aqueous phase. The step of adding diethyl ether can optionally include adding a reagent effective for quenching the reaction. In one embodiment, the reagent effective for quenching the reaction is a phosphate buffer. Again, because water will be removed in subsequent steps, it is generally desirable to limit the total amount of water introduced into the system to a relatively small amount, e.g., about 25-33 percent of the volume of the combined solution comprising the MIDA boronate, its organic solvent, and the aqueous solution of NaOH. In one embodiment, the phosphate buffer is added in an amount approximately equal to the volume of the aqueous solution of NaOH.

In certain embodiments, the present invention relates to any one of the aforementioned methods, further comprising the step of contacting the organic phase with one or more drying agents selected from the group consisting of magnesium sulfate, diatomaceous earth, and molecular sieves, thereby drying the organic phase comprising the boronic acid and free MIDA ligand. A diatomaceous earth can be, for example, Celite® (Fluka/Sigma-Aldrich, St. Louis, MO; Celite Corp., Lompoc, CA).

In certain embodiments, the present invention relates to any one of the aforementioned methods, further comprising the step of deoxygenating the dried organic phase comprising the boronic acid and free MIDA ligand. In one embodiment, the deoxygenation is accomplished by bubbling dry oxygen-free gas through the organic phase

comprising the boronic acid and free MIDA ligand. In one embodiment, the oxygen-free gas is argon.

AUTOMATED SMALL MOLECULE SYNTHESIZERS

With robust and general methods for the purification and deprotection of MIDA boronates in hand, an apparatus with the capacity for fully-automated synthesis of small molecules via ICC (**Figure 1D**) was designed and built. In certain embodiments, this apparatus is comprised of three modules, each designed to promote a deprotection (D), cross-coupling (CC), or purification (P) step required to execute the ICC scheme depicted in **Figure 1B**. In certain embodiments, all materials are transferred between modules as solutions manipulated by a series of main syringe pumps (e.g., eight) coordinated with a suite of switchable valves (J-KEM Scientific). In certain embodiments, all of the syringe pumps are driven by a computer running a custom-made software program. One embodiment of the machine is depicted in **Figure 1D**; additional details regarding this machine are provided in the Exemplification below.

Reaction System Design. In certain embodiments, the cross-coupling reactions are run in polypropylene tubes purchased from Luknova, item #FC003012. The dimensions of the tube are 21 mm x 120 mm (ID x length). The bottom of the tube is fitted with a 21 mm diameter x 4 mm tall frit. On top of this frit is secured via metal wire a 13 mm diameter x 4 mm tall frit. On top of the frit is placed a large stir bar containing a rare-earth magnet (Big Science Inc., SBM-1508-REH). The bottom of the tube is accessed through a male Luer tip, while the top of the tube is sealed with an air-tight, threaded cap containing a female Luer port. The tube holds a solvent volume of up to 25 mL. The tubes are placed in an aluminum heating block that was custom fabricated. The heating block holds up to nine reaction tubes. The tubes are held 3 cm above the surface of the stir plate, where the bottom 4 cm of the tube is jacketed by the heating block. The tubing to access the bottom of the reaction tube goes through a hole in the side of the block near the bottom.

The use of a polypropylene tube appears to be important in simplifying the engineering of the reaction tube. Specifically, the material is a good insulator such that only the portion that is jacketed by the heating block becomes hot. When the heating block is heated to 60 °C, the reaction solution reaches 60 °C within several minutes. However, the portion of the tube that is not jacketed remains at room temperature, acting as a condenser, and thus the vapor above the solvent remains at room temperature. When other

materials such as glass were used, the portion of the tube above the heating block became hot and the solution quickly evaporated. Thus, if glass were to be used instead of polypropylene, there would need to be an additional cooling element in order to keep the solution from escaping.

In certain embodiments of the system the tubes in the reaction block are stirred constantly, regardless of whether there is solution inside the tube. This keeps the system simple since the stir plate does not need to be turned on or off, and further, the start and stop times of the reactions within the block do not need to be coordinated. However, during prolonged stirring the stir bar in the tube acts as a mortar and the frit as a pestle, such that the base becomes finely ground into the pores of the frit. Further, the stir bar may be damaging the top of the frit even in the absence of base. In these situations it becomes nearly impossible to withdraw solutions through the frit since the top surface of the frit is clogged and/or damaged. To overcome this limitation a reaction tube was designed to contain two frits of different sizes (**Figure 8**). This way the stir bar only contacts the smaller top frit, and even if the top surface of this frit is damaged or clogged, the solution can be withdrawn through the sides of the small frit or through the spaces open only to the larger frit. The wire is necessary to secure the top frit so that it does not rotate sideways during the reaction to become perpendicular to the larger frit. In certain embodiments, a single frit could be fabricated to have a shape similar to the combined frits.

Purification System Design. The chromatographic properties of MIDA boronates that enable the simple purification approach are discussed above. Below is described how the engineering of the system supports the catch-and-release chromatography and precipitation-based purification.

Because diluting the crude THF reaction solution with hexanes will cause immediate precipitation of the MIDA boronate product, the mixing of THF and hexanes must occur in a container of sufficient volume to hold the precipitated product. The solvents must also be thoroughly mixed so that the solution is homogeneous. Finally, the addition of hexanes to the THF solution also causes reaction byproducts to become insoluble. In some cases these byproducts are sticky or form a very fine precipitate that can clog a frit. In certain embodiments, a custom designed precipitation chamber in which to mix the crude THF solution and hexanes can be used (**Figure 9**). In certain embodiments, the precipitation chamber contains Celite® which scavenges the sticky impurities that precipitate and keeps this material distributed throughout the Celite® so it does not clog the

frit. In certain embodiments, a stir bar in the chamber ensures proper mixing. However, it was observed that if the stir bar stirs continuously for several hours, the Celite® in the tube becomes so finely ground that it can pass through the frit and clog downstream processes. To solve this problem, after the precipitation chamber is filled with solvent and mixed, the solvent is withdrawn so that the stir bar becomes imbedded in the dry Celite® and does not stir. In other words, the stir bar can be made to stir only when it is needed – i.e., when there is solvent in the precipitation chamber – and, therefore, this process does not require turning the stir plate on/off or coordinating the stirring of other processes that use the same stir plate. In certain embodiments, 3-aminopropyl-functionalized silica gel is placed in the precipitation chamber to scavenge palladium from a crude reaction solution.

In certain embodiments, the precipitation chamber is first filled with hexanes via the auxiliary pump (thus wetting the SiO_2 column with hexanes in the process). Then, a THF reaction solution is added from the top. The main pump does not handle both the hexanes and the THF solution because residual hexanes in the syringe might cause the MIDA boronate product to precipitate in the syringe. Further, the main pump does not withdraw the waste THF:hexanes solution for this reason and to reduce contamination. Thus, once the solvents are mixed in the precipitation chamber, the solution is withdrawn by the auxiliary pump through the SiO_2 column and then sent to waste. Next, Et_2O with MeOH is added to the precipitation chamber, mixed, and then withdrawn through the SiO_2 column and sent to waste. This process is repeated with Et_2O. At this point the pure MIDA boronate product remains as a precipitate in the precipitation chamber or is at the very top of the SiO_2 column. The auxiliary pump then injects THF through the bottom of the SiO_2 column, out through the top and through to the precipitation chamber. Thus, it does not matter if the MIDA boronate product is initially in the SiO_2 column or in the precipitation chamber, since it will be dissolved in THF at either location. This system reduces the amount of THF used to elute/dissolve the product, allowing the solution to be used in the next reaction without further concentration. After the THF has been mixed in the precipitation chamber for about 30 minutes, it is withdrawn by the main pump through the 3-way union without again passing through the SiO_2 column.

Some important features of this setup are: the precipitation event occurs in a mixing chamber; the mixing chamber contains scavengers (Celite® and functionalized silica gel); the stirring can be controlled in a simple way; the precipitation chamber and SiO_2 column

are spatially separated; and the configuration of pumps allows solvents to be added and withdrawn at various junctions throughout the process.

Software. The software that controls the machine can be described as having three levels of complexity: the basic level, the functional level, and the developed level. The basic level represents essentially the combination of 1's and 0's that can be sent to the equipment to move the syringe pumps and valves. The functional level represents the simplest commands to move the equipment that could be understood by a person looking at the computer code. The developed level represents software specifically tailored to the automated synthesis machine that can be used by non-experts to modify how the synthesis is performed. The basic level is inherent to the equipment manufactured by Kloehn. The functional level came from source code provided by J-KEM when the equipment was purchased. The functional level is source code written in VB.NET that packages the commands of the basic level into easily executed subroutines. The developed level was custom designed and written in VB.NET based on the source code provided in the functional level.

More specifically, the machine is composed of a number of syringe pumps and valves that are OEM parts from Kloehn. The syringe pumps and valves were repackaged and sold by J-KEM as a custom piece of hardware. The equipment is controlled via a RS-485 serial port that sends and receives simple text string commands written in machine language specific to the Kloehn parts. Thus, on the simplest level the machine can be controlled by sending simple text strings such as "/xR", but these commands are unintelligible to anyone using the equipment. The equipment shipped from J-KEM came with source code written in VB.NET that provides subroutines to move the syringe pump to specific positions, change valves to specific positions, control the rate at which the syringe moves, and open/close solenoid valves. The code that was developed uses the subroutines from the code provided by J-KEM. The source code from J-KEM could be removed and the software could communicate with the equipment directly without losing functionality. However, the source code from J-KEM would not be by itself enough to run an automated synthesis. Thus, it was necessary to create software to enable the development of the automated synthesis machine. Details regarding certain embodiments of the software can be found in the Exemplification.

Automated Synthesizers. One aspect of the invention relates to an automated small molecule synthesizer comprising: (a) a deprotection module, in fluid communication with

(b) a drying and degassing module, in fluid communication with (c) a reaction module, in fluid communication with (d) a purification module; at least one pump which can move liquid from one module to another; and a computer equipped with software; wherein all of the modules are under the control of the computer.

In one embodiment, the deprotection module comprises immobilized or solid-supported base, e.g., NaOH, as described herein. In one embodiment, the deprotection module is constructed and arranged so as to perform deprotection with aqueous base, e.g., aqueous NaOH, as described herein.

In one embodiment, the purification module comprises a combined (or "hybrid") precipitation and catch-and-release module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the deprotection module comprises a deprotection chamber which comprises a first opening at the top of the chamber, a second opening at the bottom of the chamber, a first frit covering the second opening, and a solid-supported ammonium hydroxide reagent.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the deprotection chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the deprotection chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the solid-supported ammonium hydroxide reagent is a strong base anion exchange resin, e.g., Amberlite IRA-400 (OH^- form), Amberlite IRA 420 (OH^- form), Amberlite IRA 410 (OH^- form), Amberlite IRN-150, Amberlite IRA 900 (OH^- form), Amberlite IRA 904 (OH^- form), Amberlite IRA 910 (OH^- form), Amberlite A5836, Amberlyst A26 (OH^- form), Ambersep 900, Dowex-1 (OH^- form), Dowex-3 (OH^- form), Dowex 1-X4 (OH^- form), Dowex 1-I 9880, Dowex 1-I0131, Dowex 550 A (OH^- form), or Amberjet 4400.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the solid-supported ammonium hydroxide reagent is a strong base, type 1, anionic, macroreticular polymeric resin based on crosslinked styrene divinylbenzene copolymer containing quaternary ammonium groups, e.g., Amberlyst A26 (OH^- form).

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the deprotection module further comprises a source of gas; wherein the source of gas can be placed in fluid communication with the deprotecting chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying and degassing module comprises a combined drying and degassing chamber which comprises a first opening at the top of the combined drying and degassing chamber, a second opening at the bottom of the combined drying and degassing chamber, a first frit covering the second opening, and a plunger; and the drying and degassing module is in fluid communication with the deprotection module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the combined drying and degassing chamber further comprises a diatomaceous earth, e.g., Celite®.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the combined drying and degassing chamber further comprises activated molecular sieves.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the molecular sieves are 4 angstrom, 8-12 mesh.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the combined drying and degassing chamber further comprises potassium carbonate.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the fluid communication is a result of the connection of the second opening of the deprotection chamber to the second opening of the combined drying and degassing chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the combined drying and degassing chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the combined drying and degassing chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the combined drying and degassing chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the combined drying and degassing chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the

combined drying and degassing chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the combined drying and degassing chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the combined drying and degassing chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a source of argon.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the argon can be placed in fluid communication with the combined drying and degassing chamber to sparge the contents of the combined drying and degassing chamber; and the plunger prevents solids from lifting during sparging.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first opening of the combined drying and degassing chamber is vented to an inert gas atmosphere which is maintained near atmospheric pressure via venting through an oil-filled bubbler.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the argon can be placed in fluid communication with the combined drying and degassing chamber through the second opening of the tube of the combined drying and degassing chamber while the first opening of the tube of the combined drying and degassing chamber is vented to an inert gas atmosphere.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying and degassing module comprises a drying chamber and a degassing chamber; the drying chamber comprises a first opening at the top of the drying chamber, a second opening at the bottom of the drying chamber, a first frit covering the second opening, and a plunger; the degassing

chamber comprises a first opening at the top of the degassing chamber and a second opening at the bottom of the degassing chamber; the drying chamber is in fluid communication with the degassing chamber; and the degassing chamber is in fluid communication with the deprotection module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying chamber further comprises a diatomaceous earth, such as Celite®.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying chamber further comprises activated molecular sieves.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the molecular sieves are 4 angstrom, 8-12 mesh.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying chamber further comprises potassium carbonate.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the fluid communication between the drying chamber and the degassing chamber is a result of the connection of the second opening of the drying chamber to the second opening of the degassing chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the fluid communication between the drying and degassing module and the degassing chamber is a result of the connection of the second opening of the degassing chamber to the deprotection module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the drying chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the drying chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the degassing chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the degassing chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a source of argon.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the argon can be placed in fluid communication with the degassing chamber to sparge the contents of the degassing chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first opening of the degassing chamber is vented to an inert gas atmosphere which is maintained near atmospheric pressure via venting through an oil-filled bubbler.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the argon can be placed in fluid communication with the degassing chamber through the second opening of the tube of the degassing chamber while the first opening of the tube of the degassing chamber is vented to an inert gas atmosphere.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction module comprises a reaction chamber which comprises a first opening at the top of the reaction chamber, a second opening at the bottom of the reaction chamber, a first frit covering the second opening, and a stir bar; wherein the reaction module is in fluid communication with the drying and degassing module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction module comprises a third opening at the top of the reaction chamber through which a liquid can be

added to the reaction chamber without contacting the sidewalls or the bottom of the reaction chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first opening of reaction chamber is vented to an inert atmosphere.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first opening of the reaction chamber is fitted with a fritted tube to prevent fine solids from escaping from the reaction chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first opening of the reaction chamber is vented to an inert atmosphere maintained near atmospheric pressure via venting through an oil-filled bubbler.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein both the second opening and third opening of the reaction chamber are in fluid communication with the second opening of the drying and degassing chamber at the same time.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the reaction chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction chamber further comprises a second frit between the stir bar and the first frit; wherein the second frit is smaller than the first frit.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the second frit and the first frit are held together with a wire, to prevent the second frit from turning perpendicular to the first frit.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first frit is disc-shaped; the first frit has a diameter of between about 18 mm and about 24 mm; and the first frit has a height between about 2 mm and about 6 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first frit is disc-shaped; the first frit has a diameter of about 21 mm; and the first frit has a height of about 4 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the second frit is disc-shaped; the second frit has a diameter between about 16 mm and about 10 mm; and the second frit has a height between about 2 mm and about 6 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the second frit is disc-

shaped; the second frit has a diameter of about 13 mm; and the second frit has a height of about 4 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the shape of the first frit is that of a first disc on top of a second disc; wherein the diameter of the first disc is smaller than the diameter of the second disc.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the shape of the first frit prevents solids from passing through the second opening of the reaction chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction module further comprises a stir plate which turns the stir bar.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction module further comprises a heating block which can heat the contents of the reaction chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein at least a portion of the reaction chamber is jacketed by the heating block.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction chamber further comprises a transition metal salt.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the transition metal salt is adsorbed onto a solid.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the transition metal salt is palladium acetate.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the solid onto which the transition metal salt is adsorbed is cesium carbonate.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction chamber further comprises a phosphine ligand.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the phosphine ligand is adsorbed onto a solid.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the phosphine ligand is S-Phos (2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl).

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the catalyst is derived from an air-stable palladium precatalyst.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the solid onto which the phosphine ligand is adsorbed is cesium carbonate.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction chamber further comprises a base.

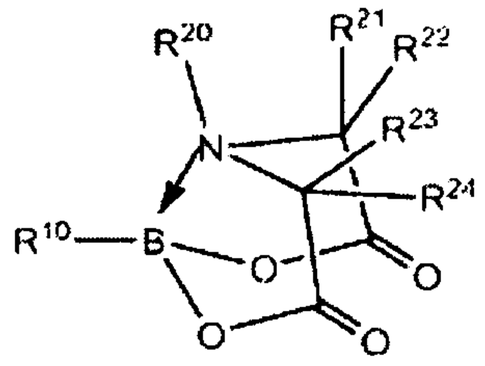
In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the base in the reaction chamber is potassium hydroxide.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction run in the reaction chamber is a cross-coupling reaction.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction run in the reaction chamber is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the reaction run in the reaction chamber is a Suzuki-Miyaura coupling.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the chemical reaction run in the reaction chamber comprises the step of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the MIDA boronate is represented by  ; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of a hydrogen group and an organic group.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module comprises a precipitation chamber and a silica column; the precipitation chamber comprises a first opening at the top of the precipitation chamber, a second opening at the bottom of the precipitation chamber, a first frit covering the second opening, a stir bar, and a diatomaceous earth (such as Celite®); and the silica column comprises a first opening at the top of the column, a second opening at the bottom of the column, a second frit covering the top opening of the column, a third frit covering the bottom opening of the column, and silica; wherein the purification module is in fluid communication with the reaction module.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the precipitation chamber comprises a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the precipitation chamber has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the precipitation chamber further comprises a resin which scavenges metals.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the precipitation chamber further comprises activated charcoal.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module further comprises a stir plate which turns the stir bar.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the diatomaceous earth (e.g., Celite®) in the precipitation chamber prevents the stir bar from turning if there is no solvent in the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the first frit of the precipitation chamber keeps the diatomaceous earth in the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the silica column is a cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column is a polypropylene cylindrical tube.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column has a length of between about 100 mm and 140 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column has a length of about 120 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column has an interior diameter of between about 18 mm and about 24 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column has an interior diameter of about 21 mm.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the cylindrical tube of the silica column has a volume of about 25 mL.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the second frit of the silica column and third frit of the silica column keep the silica in the silica column.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the silica is functionalized with amino groups.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module further comprises an auxiliary pump.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a solvent reservoir containing hexane.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module is in fluid communication with the reservoir of hexane; and the auxiliary pump provides hexane from the reservoir to the precipitation chamber by passing the hexane into the silica column through the second opening of the silica column, out of the silica column through the first opening of the silica column, and into the precipitation chamber through the second opening of the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the auxiliary pump can remove the hexane by passing the hexane into the second opening of the precipitation chamber, through the first opening of the silica column and out the second opening of the silica column.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a solvent reservoir containing diethyl ether.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module is in fluid communication with the reservoir of diethyl ether; and the auxiliary pump provides diethyl ether from the reservoir to the precipitation chamber by passing the diethyl ether into the silica column through the second opening of the silica column, out of the silica column through the first opening of the silica column, and into the precipitation chamber through the second opening of the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the auxiliary pump can remove the diethyl ether by passing the diethyl ether into the second opening of the precipitation chamber, through the first opening of the silica column and out the second opening of the silica column.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a solvent reservoir containing diethyl ether containing 1.5% methanol by volume.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module is in fluid communication with the reservoir of diethyl ether containing 1.5% methanol by volume; and the auxiliary pump provides diethyl ether containing 1.5% methanol by volume from the reservoir to the precipitation chamber by passing the diethyl ether into the silica column through the second opening of the silica column, out of the silica column through the first opening of the silica column; and into the precipitation chamber through the second opening of the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the auxiliary pump can remove the diethyl ether containing 1.5% methanol by volume by passing the diethyl ether containing 1.5% methanol by volume into the second opening of the precipitation chamber, through the first opening of the silica column and out the second opening of the silica column.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a waste container; wherein the second opening of the silica column is in fluid communication with the waste container.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, further comprising a solvent reservoir containing THF.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the purification module is in fluid communication with the reservoir of THF; and the auxiliary pump provides THF

from the reservoir to the precipitation chamber by passing the THF into the silica column through the second opening of the silica column, out of the silica column through the first opening of the silica column, and into the precipitation chamber through the second opening of the precipitation chamber.

In certain embodiments, the present invention relates to any one of the aforementioned automated small molecule synthesizers, wherein the second opening of the precipitation chamber can be placed in fluid communication with the first opening of the silica column via a three-way valve; wherein a first port on the valve is connected to the second opening of the precipitation chamber, a second port on the valve is connected to the first opening of the silica column, and a third port on the valve can be used to withdraw a solution from the precipitation chamber without passing the solution through the silica column.

Another aspect of the invention relates to any one of the aforementioned automated small molecule synthesizers, comprising: one or more deprotection modules; one or more drying and degassing modules; one or more reaction modules; one or more purification modules; at least one pump which can move liquid from one module to another; and a computer equipped with software; wherein all of the modules are under the control of the computer.

Another aspect of the invention relates to any one of the aforementioned automated small molecule synthesizers comprising: a plurality of deprotection modules; a plurality of drying and degassing modules; a plurality of reaction modules; a plurality of purification modules; at least one pump which can move liquid from one module to another; and a computer equipped with software; wherein all of the modules are under the control of the computer.

Alternative Embodiment Using Aqueous Deprotection Module. The aqueous deprotection module consists of equipment necessary to complete a solution-phase aqueous base-mediated MIDA boronate deprotection, a separation of the resulting biphasic mixture, a predrying and drying of the organic layer (e.g., ethereal solution of the boronic acid), and a deoxygenation/concentration of the dried organic layer in preparation for a cross-coupling.

Specifically, two syringe pumps and an argon solenoid are utilized in the new deprotection module (**Figure 9**). The primary syringe pump, which is used for the majority

of liquid handling during an entire sequence, handles organic solvents and solutions. A dedicated aqueous syringe pump is utilized to handle all aqueous reagents (water, 0.5 M, pH = 6, potassium phosphate buffer, and 50% saturated sodium chloride). This isolation of aqueous solutions to a dedicated syringe pump minimizes water contamination throughout the rest of the machine. The argon solenoid is used to deliver a flow of dry argon for agitating the deprotection and for concentrating and deoxygenating the final solution of boronic acid.

For example, deprotection at the beginning of a multistep sequence begins with solid MIDA boronate (1 mmol, 1 equiv) and solid sodium hydroxide (3 mmol, 3 equivs) in a 12-g Luknova cartridge. The primary pump delivers dry deoxygenated THF (10 mL, 0.1 M) to dissolve the MIDA boronate. The aqueous pump then delivers deionized water (3 mL, 0.33 M), creating a biphasic mixture, to dissolve the sodium hydroxide. A flow of dry argon is then delivered (in short 0.5-2 second pulses) from the bottom of the tube, bubbling through and agitating the biphasic mixture for 10 minutes at which time the deprotection is complete. Then, simultaneously the aqueous pump delivers phosphate buffer (3 mL) to quench the reaction, and the primary pump delivers diethyl ether (5 mL) to prepare for the separation. The aqueous pump then agitates the quenched reaction with several injections of atmospheric air. The aqueous pump then aspirates the biphasic reaction mixture, pauses to allow full separation, and returns the remaining organic layer to the deprotection tube. The aqueous layer is injected to waste and the aqueous pump delivers 50% saturated sodium chloride (3 mL) to the deprotection tube and agitates the mixture with several injections of air. Again, the aqueous pump aspirates the biphasic mixtures, pauses to allow full separation, and returns the organic layer to the deprotection tube. The aqueous layer is injected to waste.

This separation has been shown to be reproducible in the production of aqueous layer volumes. On a 1 mmol scale the first aqueous layer is 6.0 mL (± 0.1 mL). On a 0.66 mmol scale the first aqueous layer is 6.2 mL (± 0.1 mL). On a 0.33 mmol scale the first aqueous layer is 6.4 mL (± 0.1 mL). The aqueous salt volume has been shown to be 3.8 mL (± 0.1 mL) regardless of reaction scale.

Subsequent deprotections begin with the purified MIDA boronate as a solution in THF (from the automated purification) being injected into a new deprotection tube containing sodium hydroxide. The remainder of the deprotection proceeds as described above. For these downstream deprotections the only difference in experimental setup is the

amount of sodium hydroxide used (the stoichiometry is always 3 equivalents with respect to the MIDA boronate). The solvent and reagent volumes remain the same and are as outlined above. The separation volumes have shown a scale dependency and are as outlined above. The remaining manipulations (predrying, drying, and deoxygenating and concentration) proceed as described below. The relative volumes and quantities of solvents and reagents are independent of reaction scale.

Predrying of the still-wet organic layer (ethereal solution of boronic acid) removes the bulk of the remaining water. In one embodiment, the predrying tube is a 12-g Luknova cartridge containing a mixture of Celite® (800 mg) and anhydrous magnesium sulfate (2.1 g). A 5-mL polypropylene syringe plunger is placed on top of the solid mixture. The two solids are mixed intimately to prevent clumping of the magnesium sulfate clathrate. Additionally, the syringe plunger prevents movement of the solids up the tube as liquids are injected. To begin the predrying step, the primary pump delivers 5 mL of dry deoxygenated THF to the predrying tube. It has been shown that the solids adsorb approximately 5 mL of THF during this process, so wetting the solids with clean THF prevents loss of volume. Next, the primary pump transfers the organic layer from the deprotection tube into the predrying tube. The solution is passed over the solid mixture by repeated aspiration/injection (rate = 15 mL/min) via the primary pump. In total, the solution is agitated in this manner 20 times. At this point the bulk water has been removed from the solution of boronic acid.

Drying of the ethereal boronic acid solution is required to remove the remaining residual water. In one embodiment, the drying tube is a 12-g Luknova cartridge containing a layer of Celite® (300 mg) topped with activated molecular sieves (4Å, -325 mesh, 3.6 g). A 5-mL polypropylene syringe plunger is placed on top of these solids. The bottom layer of Celite® prevents clogging of the tube frit by the fine molecular sieves. The syringe plunger prevents movement of solids as described above. To begin the drying step, the primary pump delivers 5 mL of dry deoxygenated THF to the predrying tube. As described above, this prevents loss of volume. Next, the primary pump transfers the predried solution of boronic acid from the predrying tube to the drying tube. Similar to the agitation method described above, the solution is passed over the solids by repeated aspiration/injection (rate = 5 mL/min) via the primary pump. In total, the solution is agitated in this manner 12 times. The rate of aspiration during the drying step achieves appropriate aspiration and thereby thorough agitation. Specifically, a slow aspiration rate of about 5 mL/min

efficiently aspirates the boronic acid solution through the layer of molecular sieves. Faster rates result in the build-up of vacuum that is dissipated by solvent evaporation rather than solution aspiration; boronic acid solution is not efficiently passed over the molecular sieves and remains wet. At this point the boronic acid solution has been thoroughly dried.

Deoxygenating the boronic acid solution is required in preparation of the cross-coupling reaction. Specifically, the solution needs to be deoxygenated for the coupling to proceed productively. Additionally, concentration of the solution is useful to remove any diethyl ether that is still present from the deprotection workup, as well as to maintain a workable volume for the cross-coupling reaction. Workable relative volumes for the coupling reactions have been determined to be 9 mL of boronic acid solution for all couplings, regardless of reaction scale. As an exception, the final reaction in a sequence requires a boronic acid solution of 2 mL. In one embodiment, the concentration tube is an empty 12-g Luknova cartridge. To begin the deoxygenating/concentration step, the primary pump transfers the dry boronic acid solution to the concentration tube. Then, dry argon is bubbled through the solution to simultaneously deoxygenate and concentrate. The argon flow begins with short 0.5 second pulses and these pulses become progressively longer over the course of 3 minutes, at which point the argon flow remains on continuously. This concentration process has been shown to reduce volume at an approximate rate of 0.1 mL/min. Before concentration, the volume is 18 mL and, therefore, approximately 90 minutes of argon flow reduces the volume to 9 mL. The resulting dry, deoxygenated, concentrated solution of boronic acid is suitable for addition to an anhydrous cross-coupling reaction.

The aqueous deprotection module represents a robust and predictable method for the automation of MIDA boronate deprotection reactions. These types of aqueous conditions are known to work for many sensitive boronic acids in the context of non-automated synthesis, and, as such, this automated deprotection is expected to work reliably for a wide range of sensitive substrates. This aqueous deprotection module, however, differs from previously reported methods of MIDA boronate deprotection in several ways. The changes employed in the automated process relative to the procedure utilized in published solution-phase reactions include argon flow agitation and argon sparging deoxygenation/concentration, a three-step drying strategy, the minimization and use of specific solvent volumes, and controlled slow-rate aspiration for liquid handling.

In a non-automated MIDA boronate deprotection, agitation of the biphasic reaction is achieved with conventional stirring (magnetic stir bar and stirring plate). The aqueous deprotection module of the invention utilizes argon gas flow to agitate the deprotection reaction. As argon is passed through the frit of the deprotection tube, the resulting bubbles provide highly efficient agitation of the biphasic system. The agitation is sufficient to achieve full conversion at room temperature in 10 min (similar to non-automated conditions) without the use of a stir plate. Furthermore, the aqueous deprotection module uses argon flow to sparge and concentrate the boronic acid solution. In non-automated syntheses with stable boronic acids, the acid is typically isolated as a solid and submitted to a cross-coupling in the presence of deoxygenated solvent. In the case of unstable boronic acids, the acid is typically not isolated, but concentrated to some small volume by iterative concentrations from deoxygenated solvent. Use of argon sparging and gas flow concentration simultaneously in the automated system deoxygenates the boronic acid solution and concentrates it. This provides a coupling-ready boronic acid solution without the need to isolate a potentially unstable boronic acid.

Drying of the boronic acid solution for a non-automated synthesis typically involves drying over an anhydrous drying reagent, filtration through Celite®, and subsequent washing of the drying reagent. The use of excess drying reagent can insure complete drying, and the use of copious solvent volumes can insure quantitative recovery. This increased solvent volume presents a challenge in the context of automation. That is, all the excess volume accumulated in the automated process would need to be concentrated downstream. In order to minimize the accumulation of solvent, which is closely connected to the drying process, the automated system utilizes a cooperative three-step drying strategy and specific solvent volumes. The first of the three steps is a 50% saturated sodium chloride extraction of the organic phase of the deprotection reaction. This removes some bulk water from the organic phase and, as described above, does so with reproducible specific volumes. The second of the three steps is the predrying of the boronic acid solution over anhydrous magnesium sulfate, which removes more bulk water from the solution. The final step is the drying over molecular sieves, which removes the remaining residual water. As described above, each step uses specific predetermined solvent volumes to maintain minimized, yet reproducible solvent accumulation. This process, coupled with the use of predetermined, minimized quantities of drying agents, maximizes drying and substrate recovery while minimizing solvent accumulation.

Another key difference between previously reported methods for non-automated syntheses and the automated system of the invention is the use of controlled slow-rate aspiration for liquid handling. The decreased aspiration rate during the drying step, as described above, has enabled efficient handling of liquids. Specifically, aspiration rates above 2 mL/min during the drying step cause a build-up of vacuum in the primary syringe pump. The vacuum is then relieved by the evaporation of solvent. As a result, the boronic acid solution is not fully aspirated and is not efficiently dried over the molecular sieves. Using a decreased aspiration rate minimizes the build-up of vacuum and allows for full aspiration of the boronic acid solution. This slow-rate aspiration approach has also been applied to the aspiration of crude cross-coupling reaction mixtures.

Specific volumes and amounts disclosed above can, of course, be scaled up or down to suit larger or smaller overall scale automated machines, respectively, provided that the scaled volumes and scaled amounts remain proportional to one another.

An Exemplary Automated Coupling Cycle. An example of one complete cycle of automated coupling proceeds as follows.

Step 1. In the deprotection module, catch and selective release-based hydrolysis of a MIDA boronate yields a freshly-prepared boronic acid as a solution in THF.

In this example, the deprotection of MIDA-protected organoboronic acids via solid-supported ammonium hydroxide reagent proceeds without the use of added bulk water, thereby avoiding the need to remove bulk water prior to a subsequent anhydrous reactions (such as a cross-coupling). A range of aqueous deprotection conditions in the context of the automated synthesis were tried but the following problems were found:

- The amount of solvent required to extract the boronic acid product depended on the identity of the boronic acid. Polar boronic acids required much more solvent. Some boronic acids were too polar to be effectively extracted.
- The amount of solvent used in the extraction step would require an additional evaporation step to obtain a reasonable concentration.
- Removing the large amount of water in the organic phase required very large amounts of drying reagents that became impractical.

- Completely removing the water introduced in the deprotection step from the machine was very difficult from an engineering standpoint. Residual water persisted in the tubing and syringes.

Running the deprotection reaction with solid KOH and anhydrous THF did not proceed. Running the deprotection reaction with solid KOH and 1% water in THF was not a general solution because the two products of the deprotection reaction, N-methyliminodiacetic acid bis potassium salt and the boronate salt (the boronic acid reacts with KOH to produce the anionic boronate species), were both insoluble in THF and aggregated to cause the water and the THF to separate and the KOH to be sequestered, thus stalling the reaction. The use of Amberlyst A26(OH) resin solved all of these problems. The resin is not anhydrous since it is prepared in water and is shipped damp; one can control the amount of water that is present based on the volumes of organic solvent that are used to wash the resin. Accordingly, it is possible to produce a free-flowing resin that contains only enough water to allow the deprotection reaction to proceed, and not so much water that the resulting reaction solutions can not be easily dried with a small amount of molecular sieves. Further, residual water does not contaminate the equipment since bulk water is never added to the reactions. The problem of aggregation is solved because the N-methyliminodiacetic acid bis potassium salt produced in the deprotection reaction becomes trapped within the pores of the resin and does not aggregate with unreacted KOH or water. Often the boronate salt produced in the deprotection reaction also becomes trapped within the pores of the resin. Boronate salts trapped in the resin do not aggregate and do not stall the reaction. Further, this protocol is the only deprotection condition for MIDA boronates that does not require stirring. The reaction with Amberlyst A26(OH) proceeds to full conversion within 60 minutes with periodic air bubbling to mix the mixture, thus allowing a large number of deprotection reactions to be performed in parallel with simple equipment. The mixture (resin and THF) is then treated with dilute acetic acid to convert the boronate salt to the boronic acid. The very fine, very polar N-methyliminodiacetic acid produced in this process remains trapped in the Amberlyst resin which greatly facilitates filtration of the mixture. (Without the Amberlyst resin sequestering the N-metyliminodiacetic acid, the subsequent filtration step was found to be unreliable.)

Step 2. This boronic acid solution is then transferred to the cross-coupling module where it is added slowly to a stirred reaction mixture containing the next halogen-bearing building block, a palladium catalyst, and a solid inorganic base. Conversion of each halide

building block is maximized via: (a) using excess boronic acid (~3 equiv.) relative to halide (1 equiv.); (b) employing slow-addition or slow-release of the boronic acid to help avoid its decomposition *in situ* during the cross-coupling reaction; and (c) using Buchwald's highly effective and air-stable SPhosPd catalyst to maximize the generality, efficiency, and mild nature of the cross-coupling condition (D.M. Knapp, E.P. Gillis *J. Am. Chem. Soc.* **2009**, *131*, 6961-6963; and R. Martin S.L. Buchwald *Acc. Chem. Res.* **2008**, *41*, pp 1461–1473).

Step 3. The soluble components of the resulting crude reaction mixture are transferred to the purification module where the MIDA boronate product is purified via tandem precipitation and catch-and-release processes, as described above.

In the automated system the THF:hexane solution, Et_2O with 1.5% MeOH (v/v) solution and Et_2O solution are withdrawn from the top of the SiO_2 column and through the bottom under vacuum. This approach is different from standard chromatography in which the solution is pushed through the top of the column under pressure. Again, the unique elution properties of the MIDA boronate hold up under this modification, and this modification greatly simplifies the engineering of the purification step. In the automated system the THF is injected into the bottom of the column and flows out through the top under positive pressure. In this way the MIDA boronate, which is immobilized near the top of the column, has the least distance to be carried in the THF (has the smallest column volume) and thus the amount of THF used to elute the MIDA boronate can be minimized. It is believed that flowing solvents in opposite directions at separate times on the same column is not a standard chromatography practice.

This three-step cycle of deprotection, cross-coupling, and purification is iterated until the final building block coupling step is reached. To maximize efficiency, the final coupling reaction in each sequence is performed via *in situ* hydrolysis of the final MIDA boronate under aqueous basic conditions. "Slow-release" cross-coupling in this context can help maximize the yield of this final coupling reaction. Similar to the approach used in peptide, oligonucleotide, and oligosaccharide coupling, if the individual building blocks contain other types of protective groups, these are collectively removed using manually executed deprotection reactions prior to an automated chromatographic purification of the final product (**Figure 1B**).

PREPARATION OF SMALL MOLECULE NATURAL PRODUCTS

With this novel small molecule synthesis apparatus on the benchtop, its capacity was tested via the preparation a series of small molecule natural products, derived from various biosynthetic classes, including polyterpenes, fatty acids, and oxidative couplings, in a fully automated fashion (**Figure 2**). Details of selected reactions are provided in the Exemplification section below. In the idealized ICC approach, such small molecules are constructed using only stereospecific cross-coupling reactions to assemble iteratively collections of building blocks having all of the required functionality pre-installed in the correct oxidations states and with the required stereochemical relationships (several of the building blocks required for these syntheses are commercially available).

As the chromophore for vision in all vertebrates, the light-harvesting bacteriorhodopsin complex, and a derivative of a key vitamin in mammalian physiology, the polyterpine-derived natural product retinal is the focus of extensive investigations in chemistry, biology, physics, and medicine. Thus, fully automated synthetic access to this natural product and many of its derivatives stands to have a widespread impact on all of these areas of research. This natural product was automatically synthesized using two coupling cycles to unite building blocks $\mathbf{BB_4}$, commercially available $\mathbf{BB_5}$, and $\mathbf{BB_6}$ (**Figure 2A**).

The fluorescent lipid, β-parinaric acid is derived from fatty acid biosynthesis, and has proved to be very useful in a wide range of structural and functional studies of lipid bilayer membranes. Demonstrating that the modularity that exists in small molecules even crosses between biosynthetic classes, the same commercially available building block $\mathbf{BB_5}$ was used twice in the automated preparation of β-parinaric acid. Specifically, three iterated cycles of coupling led to the automated assembly of building blocks $\mathbf{BB_7}$, $\mathbf{BB_5}$, $\mathbf{BB_5}$, and $\mathbf{BB_8}$ to efficiently prepare this small molecule target (**Figure 2B**).

In another example, the neolignan ratanhine (A. Arnone, V. Di Modugno, G. Nasini, O.V. de Pava *Gazz. Chim. Ital.* **1990**, *120*, 397-401) is a modular natural product derived from oxidative coupling of common building blocks, in this case phenylpropanoid building blocks derived from phenylalanine (S.R. Angle, K.D. Turnbull, *J. Org. Chem.* **1993**, *58*, 5360-5369). Like the other derivatives, this building block-based biosynthesis strategy results in substantial modularity. For example, the propenyl fragment present in MIDA boronate $\mathbf{BB_9}$ appears in a wide range of natural products, and this building block is now

commercially available. Ratanhine was automatically synthesized via the sequential couplings of **BB_9**, **BB_{10}**, **BB_{11}**, and **BB_{12}**, followed by a final global deprotection of the methoxymethyl (MOM) ethers. This type of global deprotection is analogous to that used to deprotect automatically synthesized peptides, oligonucleotides, and oligosaccharides. See **Figure 2C**.

As shown in **Figure 2D**, the interesting PK/NRPS-derived natural product crocacin C will be automatically assembled starting with commercially-available MIDA boronate **BB_1**. Two cycles of automated couplings with **BB_2** and **BB_3** are anticipated to yield the natural product.

It is also proposed that the simple and flexible nature of automated ICC might enable the rapid preparation of derivatives of a specific small molecule with the potential to drive fundamental studies of its function. Specifically, as described above, due to its exceptional importance across a broad range of scientific disciplines, retinal has been intensely studied by chemists, biologists, biophysicists, and molecular engineers. Double bond stereochemistry is intimately linked to the function of this natural product, and probing these relationships requires efficient access to these stereoisomers in chemically pure form. Moreover, retinal derivatives that are site-selectively labeled with ^{13}C atoms are very valuable for biophysical studies mediated by NMR spectroscopy. It is recognized that the ICC approach could potentially address both of these synthetic challenges simply by incorporating into the automated synthesis sequence described above alternative MIDA boronate building blocks having the desired stereochemistry and site-specific ^{13}C labeling pre-installed (**Figure 3A**). Specifically, the fully-automated iterative coupling of various combinations of building blocks **BB_4**, **(*E*)-BB_5**, **(*Z*)-BB_5**, **(*E*)-$^{13}C_2$-BB_5**, **(*Z*)-$^{13}C_2$-BB_5**, **BB_6**, and **$^{13}C_2$-BB_6** is anticipated to yield six different derivatives of retinal, representing two possible stereoisomers, each ^{13}C-labeled in three distinct patterns (**Figure 3B**).

Automated ICC also has the theoretical capacity to substantially accelerate the preparation of many derivatives of biologically active small molecule natural products with the goal of improving their potential for medicinal applications. As a specific example, ratanhine represents just one member of a very large family of neolignan natural products. Many of these natural products demonstrate interesting antifungal, antileishmanial, antiangiogenic, antirheumatic, antitumoral, and/or hypolipidemic properties, and several have been used as lead compounds for the development of new medicines (S. Apers, A. Vlietinck, L. Pieters, *Phytochem. Rev.* **2003**, *2*, 201-217). To explore the potential of

automated ICC to prepare structural analogs of this family of natural products, four sets of building blocks representing the various substructures that commonly appear in many of these compounds have been preassembled. It is expected that fully-automated ICC can be employed to link these building blocks in all possible combinations to generate in protected form four neolignan natural products and 20 novel neolignan analogs (**Figure 4B**). After removing the hydroxyl protecting groups and automated HPLC purification, all of these natural products and natural product derivatives are expected to be produced in quantities suitable for biological assays.

The development of a fully automated ICC platform for small molecule synthesis represents an important step towards increasing the efficiency and flexibility with which small molecules can be prepared in the laboratory. While certain types of small molecules (for example, those possessing many Csp^2-Csp^2 linkages) are at present more amenable to this approach than others, the rapidly expanding scope of the Suzuki-Miyaura reaction, which increasingly includes Csp^3 coupling partners (M.R. Netherton, G.C. Fu, *Adv. Synth. Catal.* **2004**, *346*, 1525-1532) suggest that the potential generality of this approach is substantial. This synthesis apparatus stands to extend the power of small molecule synthesis to the non-chemist and ultimately will help shift the rate-limiting step in small molecule science from achieving syntheses to understanding function. Given that the functional capacity for small molecules likely extends far beyond that which is currently understood or utilized, the developments described herein stand to have widespread impacts in both science and medicine.

DEFINITIONS

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or

B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e., "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts

of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following, which is included merely for purposes of illustration of certain aspects and embodiments of the present invention, and is not intended to limit the invention.

Example 1 -- General Apparatus Design Principals

One example of an automated small molecule synthesizer is shown in **Figure 1D** (photograph) and **Figure 5** (schematic). This custom designed apparatus can execute the fully automated synthesis of eight small molecules simultaneously. Each synthesis consists of between one and three iterative coupling sequences, where each coupling sequence can include a deprotection step, a cross-coupling step and a purification step. The organization of the apparatus is centered on eight main syringe pumps. Each main syringe pump is dedicated to only one synthesis. These eight main syringe pumps operate independently to execute iterative coupling sequences in parallel. Resources for each synthesis are compartmentalized such that each main syringe pump does not access the resources of another main syringe pump, with the following exceptions: all solvents and all product output ports are shared by all of the main syringe pumps. Additionally, an auxiliary syringe pump is used as a shared resource for the purification steps. Another auxiliary syringe pump is used as a shared resource to handle all aqueous solutions. The custom designed software that operates the machine governs how the shared resources are distributed.

Standard Valve. The valve modules were purchased from J-KEM Scientific (part #Syr-CS4) and are connected to the controlling computer via a RS485 to USB connection. Each valve module is equipped with four eight-port stream-selecting valves (J-KEM, part

#SPDV-CS8). Each valve connects the input stream, which enters through the center of the valve, to one of eight possible output streams (ports A thru H). The location of the standard valves is shown in **Figure 5**.

Syringe Pump Valve. Each syringe pump is fitted with an eight-port stream selecting valve (J-KEM, part #SPDV8) where the input stream enters from the syringe connected immediately in front of port "E". Port "E" is partially blocked by the syringe and requires a flush-net fitting (IDEX Health and Science, part #F-358) to connect the output stream. The location of the syringe pump valves is shown in **Figure 5**.

Syringe Pumps. Syringe pumps were purchased from J-KEM Scientific (part #SYR1400-8 for P1-P8, part #SYR-1400PC for P9 and P10) and are connected to the controlling computer via a RS485 to USB connection. Each syringe pump is fitted with an eight-port stream-selecting valve (J-KEM, part #SPDV8) and a 10 mL glass syringe equipped with a Teflon plunger (J-KEM, part #SPGS-10000). The syringe pump withdraws and injects at rates from 0.0 mL/min to 70.0 mL/min with a step of 0.0029 mL. The location of the syringe pumps is shown in **Figure 5**.

Reaction Tubes. To minimize cross-contamination and allow the rapid setup of a synthesis, all chemical manipulations are performed in disposable polypropylene tubes purchased from Luknova, item #FC003012. The dimensions of the tube are 21 mm x 120 mm (ID x length). The bottom of the tube is fitted with a 21 mm diameter x 5 mm tall frit. The bottom of the tube is accessed through a male Luer tip, while the top of the tube is sealed with an air-tight, threaded cap containing a female Luer port. The tube holds a solvent volume of up to 25 mL. Examples of reaction tubes are shown in **Figure 6** and **8**.

Tubing and Fittings. All tubing and fittings were purchased from IDEX Health and Science. The tubing used in the machine is 0.030 inch (ID) x 1/16 inch (OD) Teflon FEP tubing (part #1520xL). All tubing connections were made with 1/16 inch ETFE flangeless ferrules (part #P-200x) and ¼-28 acetal fittings (part #P-202x). Male Luer fittings (part #P-625) and female Luer fittings (part #P-658) are ETFE and PEEK, respectively. Examples of tubes and fittings are shown in **Figure 6**.

Example 2 -- Computer Control of Apparatus

General Programming Design Principals. All apparatus equipment is controlled by a custom program written in VB.NET using Microsoft Visual Basic 2008 Express Edition. The apparatus is controlled by a single computer running Windows Vista. The apparatus is

designed to run an experiment independently on each of the eight main syringe pumps. Each main pump can be started and stopped at will without affecting the other pumps. Further, the scripts of simultaneously executing experiments do not need to be the same nor do they need to be synchronized in order for the experiments to perform successfully. The program is designed to manage distribution of shared resources such as common valve equipment and auxiliary syringe pumps as well as bandwidth on the COM ports.

Setting up and modifying the script used to execute an experiment is designed to be straightforward and facile. Towards this end, a simple custom scripting language was developed. The scripting language contains a small collection of commands used to send instructions to a virtual machine, manage the timing of these operations, and lock/unlock access to shared resources. The program serves as an interpreter for this custom scripting language and maps the virtual machine instructions to the required apparatus hardware. In this way the same script can be used on any of the main syringe pumps without modification.

Communication to the Apparatus Equipment. Commands are sent to the apparatus equipment using the computer's serial ports. The RS485 serial ports of the apparatus equipment are connected to a serial-to-USB converter which is connected to the computer. Each COM port can address up to 16 pieces of equipment, where a piece of equipment is defined as a valve or a syringe pump. The syringe pump may or may not additionally control a solenoid valve. As configured, communication to the apparatus is distributed across four COM ports. Commands can be sent to the equipment no faster than every 20 milliseconds. To enforce this delay, the program maintains a queue of commands to be sent to the equipment. Commands recognized by the equipment are: move the valve to a specific port, report the current position of the valve, move the syringe plunger to a specific position at a specific rate, turn the solenoid valve on/off, and report if the equipment has finished executing the previous command.

Program Architecture. Each valve and each syringe pump is represented programmatically as an "equipment object". Each of these equipment objects is given an identifier that can be used to map the commands of the scripting language to the actual hardware represented by the equipment object. Each main pump is assigned a "pump object" that is a container for all of the equipment objects that represent resources that are available to the main pump. No single pump object contains every equipment object, but every equipment object is contained in at least one pump object. When an experiment is

executed on the main pump, the list of scripted commands is passed as a text file to the corresponding pump object. The pump object is responsible for proof-reading, interpreting and executing the scripted commands and updating the graphic user interface (GUI) as appropriate.

Script Execution. When the pump object is passed a text file of command lines, the pump object interprets each command line into a "command object". The command object contains all of the information that is necessary to execute the command. Once a command object is created it is entered into a queue managed by the pump object. Through this process the script is proof-read to identify any syntax errors that would prevent the code from executing properly. The script is then executing by retrieving an item from the queue, sending the appropriate commands to the equipment via the COM port, periodically checking the status of the machine equipment until the command is complete, and then repeating the process for the next command in the queue.

Scripting Language. The scripting language contains the following commands with indications on their usage:

' (apostrophe)	Indicates everything following the apostrophe is a comment and should not be interpreted.
[*text*] (brackets)	The brackets represent a placeholder value recognized by the identifier *text* that will be replaced at runtime with the **define** command.
define *text=value*	At runtime replaces all brackets (placeholders) containing *text* with the value of *value*. This a useful strategy for writing flexible scripts.
pause *n*	Halts execution of the script for *n* seconds.
valve *nL*	Moves valve *n* to port position *L* (A-H).
valve xon **valve xoff**	**valve xon** opens the solenoid valve associated with the main syringe pump. **valve xoff** closes this solenoid valve.
pump *n* **in**=*L* **out**=*M* **ratein**=*x* **rateout**=*y*	Fills the syringe with *n* mL drawn from port position *L* (A-H) at a rate of *x* mL/min. and inject *n* mL out through port position *M* (A-H) at *y* mL/min. The port position refers to the valve that is directly connected to the syringe. Omitting the **in**= portion instructs the pump to only dispense *n* mL as per above. Omitting the **out**= portion instructs the pump to only fill *n* mL as per above.

	Use **rate**=*x* to set both the rate of withdrawal and the rate of injection to *x* mL/min.
pump out=*M* **rate**=*x*	Injects the entire contents of the syringe out through port position *M* (A-H) at a rate of *x* mL/min.
log "*comment text*"	Writes a time-stamped entry to the log book containing the user-defined text (*comment text).*
lock *n*	Claims valve *n* for the exclusive use by the main pump. If valve *n* is busy or has been locked by a different pump, execution of the script is halted until the valve becomes available. Auxiliary pumps can also be locked using this command.
unlock *n*	Releases valve *n* from the exclusive use by the main pump. All **lock** commands should be eventually followed by an **unlock** command. Auxiliary pumps can also be released using this command.
sub *sub_name* **end sub**	The **sub** and **end sub** commands mark the beginning and end, respectively, of command lines that will be interpreted as a sub routine with the identifier *sub_name.*
run *sub_name*	Runs the sub routine identified as *sub_name.* This sub routine should have been previously defined using the **sub** and **end sub** commands. The command following the **run** command is not executed until all of the commands in the sub routine have completed (as opposed to the **background** command.)
background *sub_name*	Runs the sub routine identified as *sub_name.* This command is similar to the run command except that as soon as the sub routine begins to execute, the command following the **background** command executes as well. Therefore, the sub routine is handled as a background process allowing multiple actions to be performed at once.
wait *sub_name*	Halts execution of the script until the sub routine identified as *sub_name* (which was previously executed using the **background** command) finishes its execution. This command allows background processes to be synchronized with the main script.

Example 3 -- Chemical Synthesis

General Procedure. All chemical manipulations were performed in polypropylene tubes purchased from Luknova, item #FC003012. The dimensions of the tube are 21 mm x

120 mm (ID x length). The bottom of the tube is fitted with a 21 mm diameter x 5 mm tall frit. The bottom of the tube is accessed through a male Luer tip, while the top of the tube is sealed with an air-tight, threaded cap containing a female Luer port. The tube holds a solvent volume of up to 25 mL.

Deprotection Tubes. To enable automation, a novel MIDA boronate deprotection method was developed using Amberlyst A26(OH) resin. Amberlyst A26(OH) was purchased from Sigma-Aldrich and was stored under N_2 atm. at 4 °C. Amberlyst A26(OH) (suspension volume of 20 mL) was twice washed with MeCN (50 mL) with vigorous agitation for 60 seconds in each wash. The residual solvent was evaporated under a fast stream of air for 5 minutes until the resin was light beige in color and was free-flowing. To each polypropylene tube was added the Amberlyst resin (2.0 g resin for every 1.0 mmol of MIDA boronate to be deprotected) and, optionally, the MIDA boronate starting material. The tube was capped and then placed on the machine where the bottom Luer tip connected to the deprotection table and the top Luer port is covered with aluminum foil.

Drying and Degassing Tubes. A polypropylene tube was charged with Celite®, activated molecular sieves (4Å, 8-12 mesh) and K_2CO_3. The amounts of these reagents are proportional to the amount of Amberlyst A26 resin used in the deprotection step prior to drying/degassing, as indicated below. Onto the bed of solids was placed a plastic plunger, cut from the plunger of a 5 mL polypropylene syringe (Fisher #14-817-28, Norm-Ject). The plunger prevents the solids from lifting during the degassing step. The tube was capped and then placed on the machine where the bottom Luer tip connects to the degassing table and the top Luer port is connected to the gas manifold.

Amberlyst A26 (previous step)	Celite®	Mol. sieves	K_2CO_3
2.0 g	200 mg	2.0 g	2.0 g
1.0 g	100 mg	1.0 g	1.0 g
0.5 g	50 mg	0.5 g	0.5 g

Reaction Tubes. To assist in the transfer of small amounts of $Pd(OAc)_2$ and S-Phos, these reagents were adsorbed onto Cs_2CO_3 as follows. To a 40 mL glass vial was added $Pd(OAc)_2$ (22 mg) and Cs_2CO_3 (2.723 g). To the vial was added THF (10 mL), and the suspension was concentrated in vacuo to afford a pale amber powder, the Pd-mixture. To a 40 mL glass vial was added S-Phos (76 mg) and Cs2CO3 (2.667 g). To the vial was added

THF (10 mL), and the suspension was concentrated in vacuo to afford a white powder, the SPhos-mixture.

To a polypropylene tube was added a stir bar (Big Science Inc., SBM-1508-REH), the halide (0.333 mmol), the Pd-mixture (488 mg, 5% Pd) and the SPhos-mixture (488 mg, 10% S-Phos). For aqueous couplings, to the tube was added a KOH pellet (75 mg, 1.7 mmol). The tube was capped with a modified cap (see detail) and was placed in a heating block. The bottom of the tube is connected the reaction table, the top of the tube is vented to the gas manifold, and the second top input is connected to the reaction table for addition of the boronic acid. Building on the previously published reports of "slow-release" cross-coupling, the boronic acid was added slowly via syringe pump to minimize *in situ* decomposition and thereby maximize yields.

Automation. Each cross-coupling in the automated sequence was performed according to the following, fully automated script:

Deprotection

1) Add THF (5 mL) to deprotection tube
2) Agitate the mixture via gas bubbling for 60 minutes
3) Add AcOH in THF (4.0 M, 5.0 mmol per 1.0 g of Amberlyst resin)
4) Agitate the mixture via gas bubbling for 15 minutes
5) Transfer the solution to the drying tube, washing the resin with THF (5 x 1.0 mL)
6) Sparge the mixture with Ar gas for 15 minutes
7) While sparging the reaction tube with Ar gas for 15 min., agitate the THF mixture via gas bubbling every 2 minutes

Cross-Coupling

8) Add THF (3 mL) to the reaction tube and allow the mixture to stir for 10 min.
9) Transfer the boronic acid solution from the drying tube to the reaction tube over 120 min., washing the solids with THF (3 x 1.0 mL)
10) Stir the reaction mixture at 150 rpm for 22 hours.

Purification

11) Add hexanes (12 mL) to the ppt. chamber, then add a portion of the reaction solution (3 mL) to the ppt. chamber.

12) Withdraw the solution in the ppt. chamber through the SiO_2 plug and send to waste

13) Repeat steps 11 and 12 until all of the reaction solution has been transferred

14) Add Et_2O w/ MeOH (1.5 % v/v) (7.5 mL) to the ppt. chamber, withdraw the solution through the SiO_2 plug, and send to waste. Repeat an additional four times.

15) Add Et_2O (7.5 mL) to the ppt. chamber, withdraw the solution through the SiO_2 plug, and send to waste. Repeat an additional two times.

16) Flow Ar gas through the SiO_2 plug for three minutes to evaporate residual solvent.

17) Add THF (6.8 mL) to the ppt. chamber

18) Withdraw the THF solution through the SiO_2 plug and then inject the solution back into the ppt. chamber. Repeat an additional two times.

19) Withdraw the THF solution through the SiO_2 plug and inject the solution into the deprotection tube used in the next reaction. The next reaction begins at step 1.

Direct release/aqueous coupling modification (typically performed as the last step of an automated synthesis). This sequence begins after step 19 from the general automation script (above).

1) Sparge the THF solution derived from the purification of the previous cross-coupling with Ar gas for 15 minutes.

2) Transfer the THF solution to the reaction tube in one portion.

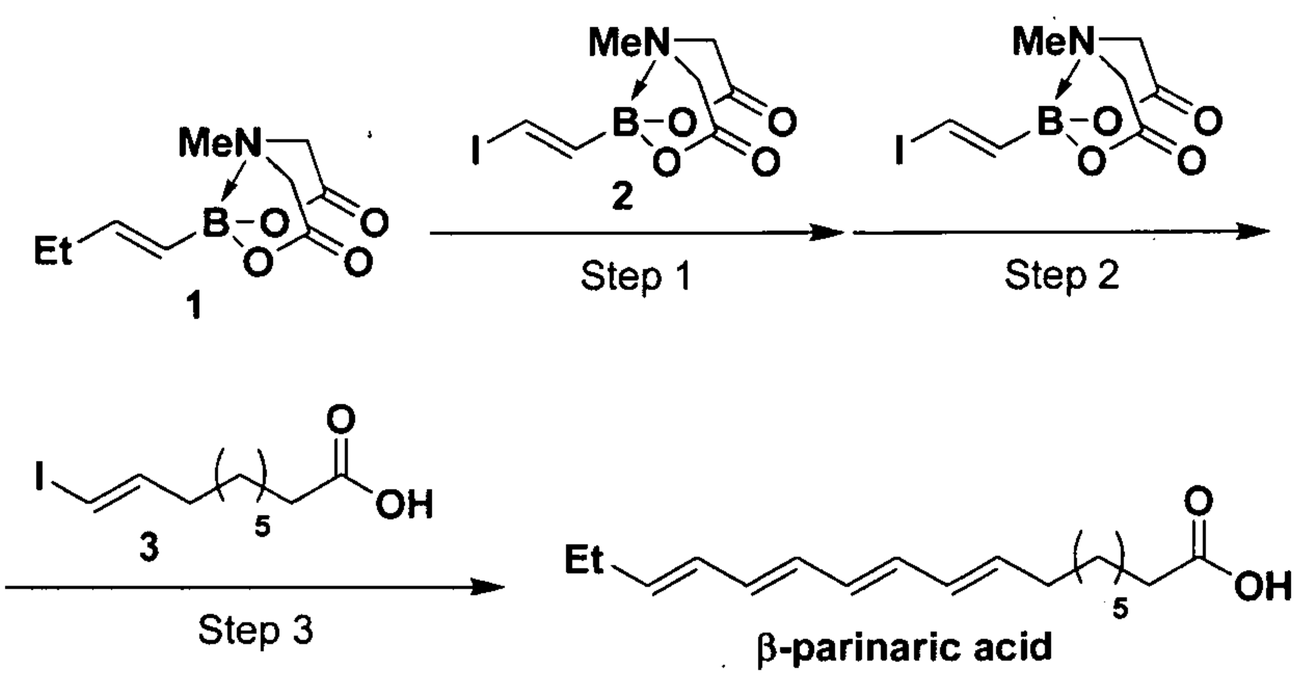
3) Stir the mixture for 5 minutes.

4) Add degassed H_2O (2 mL) to the reaction tube

5) Stir the reaction mixture at room temperature for 12 hours.

6) Add aq. NH_4Cl (2.5 mL), mix for 5 minutes, then withdraw the entirety of the mixture and transfer it to the product test tube.

β-parinaric acid. All steps were performed according to the general procedure. The machine was equipped with reagent tubes as follows.



Step 1. The machine was equipped with a deprotection tube charged with Amberlyst resin (2.0 g) and MIDA boronate **1** (211 mg, 1.0 mmol); a drying tube charged with molecular sieves (2.0 g), K_2CO_3 (2.0 g) and Celite® (0.2 g); and a reaction tube charged with the Pd-mixture (488 mg), the SPhos-mixture (488 mg) and boronate **2** (103 mg, 0.333 mmol).

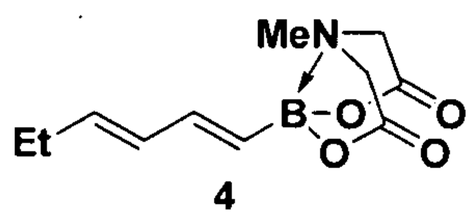
Step 2. The machine was equipped with a deprotection tube charged with Amberlyst resin (1.0 g); a drying tube charged with molecular sieves (1.0 g), K_2CO_3 (1.0 g) and Celite® (0.1 g); and a reaction tube charged with the Pd-mixture (244 mg), the SPhos-mixture (244 mg), and boronate **2** (34 mg, 0.11 mmol).

Step 3. The machine was equipped with a deprotection tube (empty, but used for sparging the MIDA boronate solution) and a reaction tube charged with the Pd-mixture (60 mg), the SPhos-mixture (60 mg), KOH (75 mg, 1.7 mmol) and halide **3** (11 mg, 0.037 mmol).

Automation. The synthesis was performed in a fully automated fashion with no operator intervention. Step 1 and step 2 were performed following the standard script, and step 3 was performed following the direct release/aqueous coupling modification of the standard script. The aqueous mixture that was outputted from the machine was manually purified as follows: The mixture was transferred to a 60 mL separatory funnel and was

diluted with sat. aq. NH_4Cl (10 mL). The mixture was extracted twice with Et_2O (20 mL) and the combined organics were washed with brine (20 mL); dried over $MgSO_4$; filtered, then concentrated *in vacuo*. The yellow residue was purified via SiO_2 chromatography to afford β-parinaric acid as a white solid (yield not yet determined). The ^{1}H-NMR ($CDCl_3$) spectrum of the synthesized product was fully consistent with the literature data (Lee, S. J.; Gray, K. C.; Paek, J. S.; Burke, M. D. *J. Am. Chem. Soc.*, **2008**, *130*, 466-468).

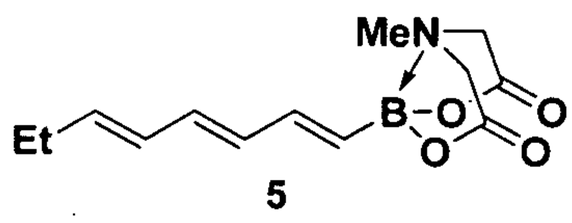
To characterize the efficiency of each step and characterize all intermediates, Step 1 was repeated and the MIDA boronate solution generated in line 19 of the standard script was diverted into a test tube and then concentrated to afford pure intermediate **4** as a colorless solid (40 mg, 52%).



4

^{1}H-NMR (500 MHz, acetone-d_6): δ 6.53 (dd, J = 17.0, 10 Hz, 1H), 6.11 (dd, J = 15.5, 10.0 Hz, 1H), 5.82 (dt, J = 15.5, 6.5 Hz, 1H), 5.54 (d, J = 17.5 Hz, 1H), 4.20 (d, J = 17.0 Hz, 2H), 4.01 (d, J = 17.0 Hz, 2H), 2.98 (s, 3H), 2.10 (quint, J = 7.5 Hz, 2H), 0.99 (t, J = 7.5 Hz, 3H). ^{13}C-NMR (125 MHz, acetone-d_6): δ 196.1, 143.7, 137.8, 132.6, 62.2, 47.2, 26.1, 13.7.

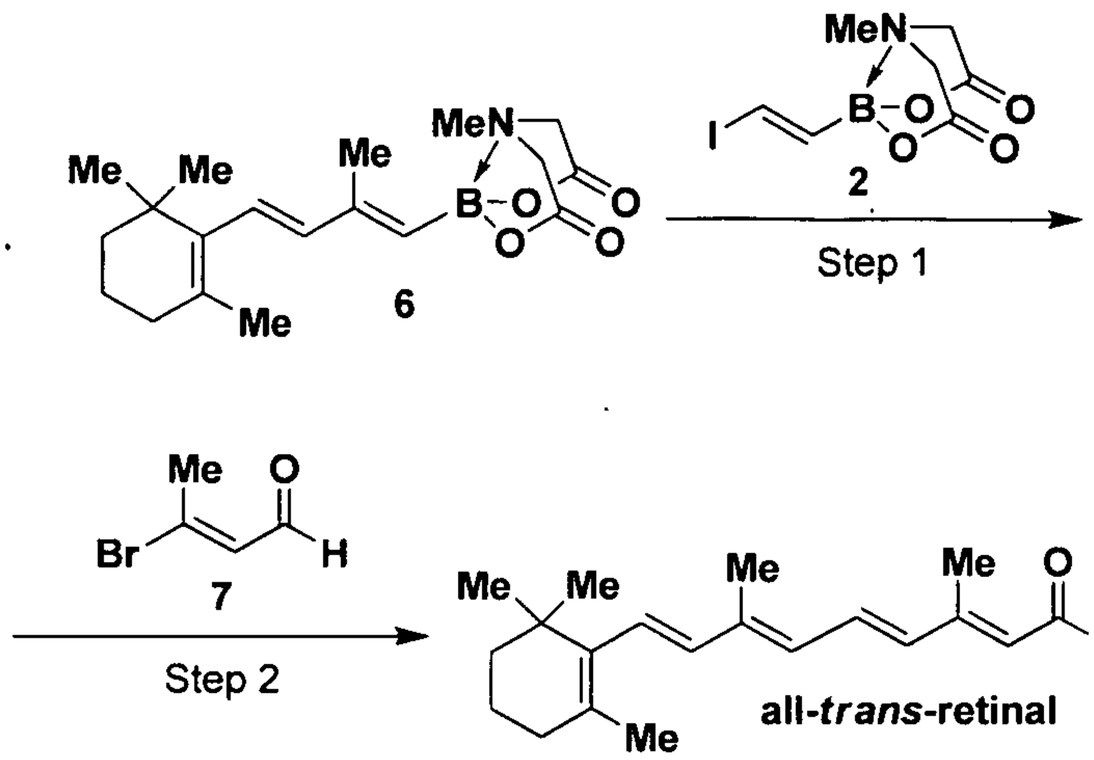
Step 1 and step 2 were repeated and the MIDA boronate solution generated during the second coupling (line 19 of the standard script) was diverted into a test tube and then concentrated to afford pure intermediate **5** as a colorless solid (22 mg, 76%).



5

^{1}H-NMR (500 MHz, acetone-d_6): δ 6.58 (dd, J = 18.0, 10.5 Hz, 1H), 6.28 (dd, J = 15.0, 10.0 Hz, 1H), 6.20 (dd, J = 15.0, 10.0 Hz, 1H), 6.11 (ddt, J = 15.5, 10.5, 1.5 Hz, 1H), 5.82 (dt, J = 15.0, 6.5 Hz, 1H), 5.64 (d, J = 17.5 Hz, 1H), 4.21 (d, J = 17.0 Hz, 2H), 4.03 (d, J = 17.0 Hz, 2H), 2.99 (s, 3H), 2.12 (quint, J = 7.5 Hz, 2H), 0.99 (t, J = 7.5 Hz, 3H). ^{13}C-NMR (125 MHz, acetone-d_6): δ 169.0, 143.5, 138.3, 134.8, 133.5, 130.3, 62.2, 47.3, 26.4, 13.8.

all-*trans*-retinal. All steps were performed according to the general procedure. The machine was equipped with reagent tubes as follows.



Step 1. The machine was equipped with a deprotection tube charged with Amberlyst resin (1.0 g) and MIDA boronate **6** (173 mg, 0.500 mmol); a drying tube charged with molecular sieves (1.0 g), K_2CO_3 (1.0 g) and Celite® (0.1 g); and a reaction tube charged with the Pd-mixture (244 mg), the SPhos-mixture (244 mg) and boronate **2** (52 mg, 0.111 mmol).

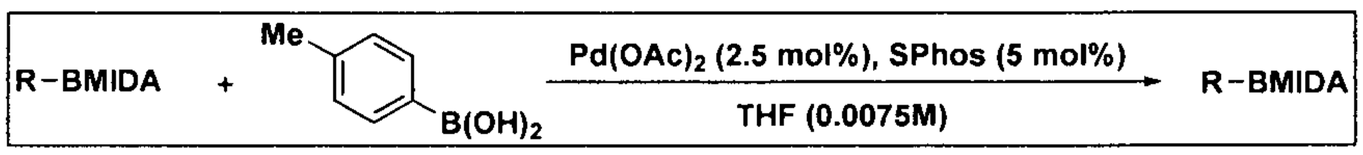
Step 2. The machine was equipped with a deprotection tube charged with Amberlyst resin (0.5 g); a drying tube charged with molecular sieves (0.5 g), K_2CO_3 (0.5 g) and Celite® (50 mg); and a reaction tube charged with the Pd-mixture (82 mg), the SPhos-mixture (82 mg). A separate polypropylene tube was charged with a solution of halide **7** (0.056 mmol) in degassed THF (3 mL).

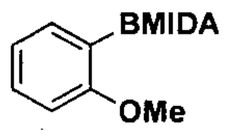
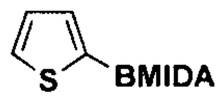
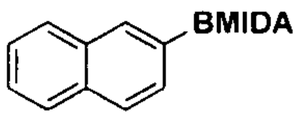
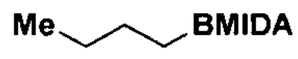
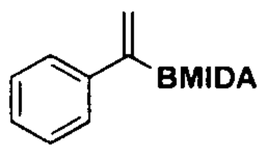
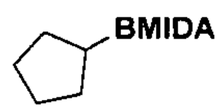
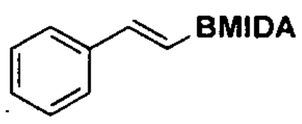
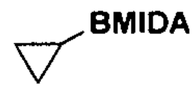
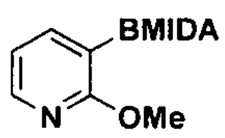
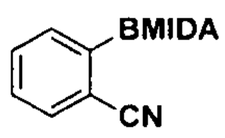
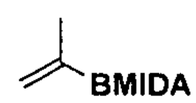
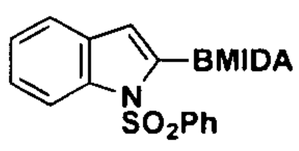
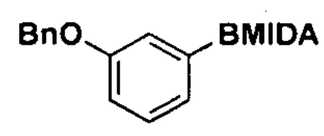
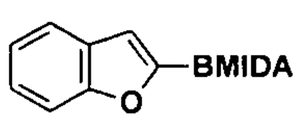
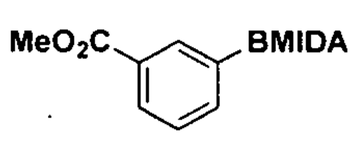
Automation. The synthesis was performed in a fully automated fashion with no operator intervention. Step 1 was performed following the standard script. Step 2 was performed following the standard direct release/aqueous coupling modification script with the additional modification that the THF used in line 8 of the standard script was the entirety of the solution containing halide **7**. Further, the script for step 2 was stopped after line 10 and the reaction solution was outputted to a test tube. The product was manually purified as follows: The reaction solution was concentrated *in vacuo* and the solid yellow residue was purified by SiO_2 chromatography using an Isco-Teledyne CombiFlash system to afford all-trans-retinal as a yellow solid (3.3 mg, 20%). The ^{1}H-NMR ($CDCl_3$) spectrum of the product was fully consistent with the literature data (Lee, S. J.; Gray, K. C.; Paek, J. S.; Burke, M. D. *J. Am. Chem. Soc.*, **2008**, *130*, 466-468).

Example 4 – Generality of Purification Platform

To establish the generality of the novel purification platform, its capacity to purify a series of MIDA boronates representing a diverse range of structures, including aryl, heteroaryl, alkynyl, alkenyl, and alkyl derivatives, was tested. Briefly, mock crude reaction mixtures were prepared by mixing each MIDA boronate (1 equiv.) (**Table 1**) with tolyl-boronic acid (1 equiv.) and a palladium catalyst (0.1 equiv) in THF. Each of these mixtures was then subjected to fully-automated purification via the hybrid precipitation/catch-and-release platform described in detail herein. At the end of this process, all of these MIDA boronates were obtained in >90% purity as judged by ^{1}H NMR (see **Figures 11-26**), and the yields of recovered MIDA boronates were good to outstanding (**Table 1**).

Table 1. Purification from mock crude reaction mixtures



Boronate	% Recovery	Boronate	% Recovery
	69		76
	53		86
	92		87
	92		90
	92		68
	83		94
	81		86
	78		65

Details of the procedure are as follows.

Pre-activation of the Catalyst Solution: Palladium(II) acetate (0.001875 mmol, 2.5 mol%) and SPhos (0.00375 mmol, 5 mol%) per purification to be run were combined in an 8 mL scintillation vial equipped with a PTFE-coated magnetic stir bar and placed under an

argon atmosphere. THF was added to generate a 0.01 M catalyst stock solution (with respect to palladium(II) acetate), and it was stirred vigorously for 30 min at room temperature to generate an orange, yellow, or clear solution. After this activation process, 1 mL catalyst stock solution was added to the solution in the polypropylene cartridge containing the simulated reaction mixture.

Preparation and Installation of Simulated Reaction Chamber: A new fritted 12 g Luknova polypropylene cartridge was charged with MIDA boronate (0.075 mmol, 1 eq), 4-methylbenzene boronic acid (0.075 mmol, 1 eq), and THF (10 mL). After addition of the pre-activated catalyst solution, the cartridge was installed into the Luer fittings in the reaction block of the automated synthesizer. Once all cartridges were in place, the automated purification routine was run using the computer interface. The samples were collected as THF solutions into tared 40 mL scintillation vials.

Concentration, Azeotropic Drying, and Analysis of Recovered Materials from Purifications: The THF solutions were concentrated under reduced pressure on a rotary evaporator, then the residue was azeotroped with dichloromethane (3 x 5 mL) to remove residual solvents. These residues were then placed under vacuum for 12-36 hours, after which yield and purity were determined by comparison of ^{1}H NMR in acetone-*d*6 with a standard sample of the desired MIDA boronate and with a sample taken of a simulated reaction mixture.

Automated Purification Detailed Protocol

1) In the background, auxiliary pump aspirates 6 mL hexanes and delivers it to the bottom of the precipitation chamber, through the silica gel column. This process is repeated once for a total of 12 mL hexanes.
2) Primary pump aspirates 9 mL of reaction mixture from reaction chamber bottom and returns 6 mL, through bottom, to ensure no more than 3 mL will be delivered to the precipitation chamber.
3) Primary pump delivers 3 mL of reaction mixture to top of precipitation chamber containing 12 mL hexanes. This induces MIDA boronate precipitation from the THF solution. Primary pump then delivers two 10-mL plugs of dry nitrogen to bottom of precipitation chamber (bypassing the silica gel column) to dislodge stir bar.

4) Suspension in precipitation chamber is aspirated from bottom and through silica gel column by auxiliary pump. Eluent is sent to waste.

5) Steps 1-4 repeat three additional times to send all of reaction mixture to precipitation chamber.

6) Primary pump aspirates 1.5 mL THF and delivers it to the top of reaction chamber as a rinse. Steps 1-3 are repeated.

7) Primary pump aspirates 1.5 mL THF and delivers it to top of reaction chamber as a rinse. Steps 2-3 are repeated.

8) Step 4 is repeated.

9) Steps 1-4 are repeated.

10) Step 4 is repeated.

11) Primary pump aspirates 6.5 mL 1.5% (v/v) MeOH in Et_2O and delivers it to top of precipitation chamber. This process is repeated once for a total delivery of 13 mL solvent.

12) Primary pump delivers two 10-mL plugs of dry nitrogen to the bottom of the precipitation chamber (bypassing the silica gel column) to dislodge stir bar.

13) Step 4 is repeated.

14) Steps 11-13 are repeated. Step 4 is repeated again.

15) Steps 11-13 are repeated twice with Et_2O instead of 1.5% (v/v) MeOH in Et_2O. Step 4 is repeated twice more to dry out silica gel column.

16) Auxiliary pump is rinsed with 2 x 1 mL THF to wash away any residual MeOH. Wash THF is sent to waste.

17) Auxiliary pump aspirates 6 mL THF and delivers slowly to bottom of precipitation chamber through silica gel column. This process is repeated once for a total of 12 mL THF.

18) Primary pump aspirates 5 mL dry nitrogen and delivers it to bottom of precipitation chamber (bypassing the silica gel column) to agitate the suspension, thus promoting mixing MIDA boronate dissolution. This process is done 40 times.

19) THF solution of MIDA boronate is aspirated by primary pump out of the bottom of the precipitation chamber (bypassing the silica gel column). Solution is delivered to the collection tube. This aspiration/delivery is repeated an additional 5 times to ensure full transfer.

20) Auxiliary pump pushes residual THF in silica gel column into bottom of precipitation chamber as a rinse.

21) Primary pump aspirates 5 mL dry nitrogen and delivers it to bottom of precipitation chamber (bypassing the silica gel column) to agitate the suspension, thus promoting mixing MIDA boronate dissolution. This process is done 5 times.

22) THF rinse is aspirated by primary pump out of bottom of the precipitation chamber (bypassing the silica gel column). Solution is delivered to the collection tube.

The results from this study of a wide range of structurally diverse MIDA boronates demonstrates that the hybrid precipitation/catch-and-release purification strategy is remarkably general.

Example 5 -- Aqueous Deprotection Module

Automated aqueous deprotection of phenyl MIDA boronate, trienyl MIDA boronate, and butenyl MIDA boronate was performed using the aqueous deprotection strategy and module described above.

Automated aqueous deprotection of phenyl MIDA boronate afforded phenyl boronic acid. Subsequent automated cross-coupling with the vinyl iodide bifunctional building block afforded the desired coupled product in 77% yield after manual purification. See **Figure 28A**.

Automated aqueous deprotection of the trienyl MIDA boronate afforded the trienyl boronic acid. Subsequent automated cross-coupling with the vinyl iodide bifunctional building block afforded the desired coupled product in 66% yield after manual purification. See **Figure 28B**.

Automated aqueous deprotection of the butenyl MIDA boronate afforded the butenyl boronic acid. Subsequent automated cross-coupling with an isomeric mixture of the dienyl vinyl iodide bifunctional building block afforded a 75% yield of the expected isomeric trienyl coupled products in a 75% yield after automated purification. See **Figure 28C**.

Example 6 -- Fully Automated Synthesis of All-*trans*-Retinal Using Aqueous Deprotection Module

The first deprotection tube was prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was added trienyl MIDA boronate (345.2 mg, 1 mmol, 9 equivs). To this was added sodium hydroxide (120.0 mg, 3 mmol, 27 equivs). The cartridge was capped with its female luer-port screw cap. To this Luer port was attached a 5-mL polypropylene syringe barrel (Henke-Sass, Wolf GmbH, Tuttlingen, Germany, 78532, Part # A5) from which the plunger had been removed. This first deprotection tube was wrapped with aluminum foil.

The second deprotection tube was prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was added sodium hydroxide (40.0 mg, 1 mmol, 9 equivs). Sodium hydroxide pellets were shaved down to the correct mass with a clean razor blade and massed quickly to minimize adsorption of atmospheric moisture. The cartridge was capped with its female luer-port screw cap. To this Luer port was attached a 5-mL polypropylene syringe barrel (Henke-Sass, Wolf GmbH, Tuttlingen, Germany, 78532, Part # A5) from which the plunger had been removed. This second deprotection tube was wrapped with aluminum foil.

The first and second predrying tubes were prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was added Celite® 545 filter aid (800 mg, not acid-washed, Acros Organics, Product # 349670025, Lot # A0287832). To this was added anhydrous magnesium sulfate (2.1 g, ReagentPlus®, ≥99.5%, Sigma-Aldrich, Product # M7506, Lot # 080M0246V). These two solids were mixed with a spatula until visibly homogenous. On top of the solid mixture was placed a 5-mL polypropylene syringe plunger (Henke-Sass, Wolf GmbH, Tuttlingen, Germany, 78532, Part # A5), manually cut to approximately 6.5 cm in length. The cartridge was capped with its female luer-port screw cap. The Luer port was covered tightly with a small square (approximately 1 cm x 1 cm) of aluminum foil. Each predrying tube was wrapped with aluminum foil.

The first and second drying tubes were prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was added Celite® 545 filter aid (300 mg, not acid-washed, Acros Organics, Product # 349670025, Lot # A0287832). To this was added activated molecular sieves (3.6 g, 4Å, -325 mesh, Sigma-Aldrich,

Product # 688363, Lot # MKBF4010V). Molecular sieves were activated at 300 °C, ambient pressure, 24 h, and cooled/stored in a vacuum desiccator under dry argon over Drierite. These two solids were not mixed. On top of the layered solids was placed a 5-mL polypropylene syringe plunger (Henke-Sass, Wolf GmbH, Tuttlingen, Germany, 78532, Part # A5), manually cut to approximately 5.5 cm in length. The cartridge was capped with its female luer-port screw cap. Each drying tube was wrapped with aluminum foil.

The first and second deoxygenating/concentrating tubes were prepared as follows: A new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was capped with its femal luer-port screw cap. Each deoxygenating/concentrating tube was wrapped with aluminum foil.

The first reaction tube was prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) was added a 4-g frit (Luknova, Mansfield, MA, 02048, Part # FC003004). This frit was secured, concentrically, to the 12-g cartridge frit with 26 G Chromel A wire, pierced through the 12-g frit. To this reaction tube was added, in order, anhydrous potassium phosphate (1.39 g, 3 mmol + 750 mg, 27 equivs + 750 mg, 97%, Alfa Aesar, Product # L15168, Lot # L02U015), palladium (II) acetate (1.9 mg, 0.0083 mmol, 2.5 mol%, ≥ 99.9%, Sigma-Aldrich, Product # 520764, Lot # 1000824996), 2-dicyclohexylphosphino-2',6'-dimethyoxy-1,1'-biphenyl (Sphos, 6.8 mg, 0.017 mmol, 5 mol%, 98%, Strem Chemicals, Product # 15-1143, Lot # 18526300), vinyl iodide MIDA boronate (103.0 mg, 0.33 mmol, 3 equivs), and a PTFE-coated rare earth magnetic stir bar. Potassium phosphate was freshly ground in a 100 °C mortar and pestle. The cartridge was capped with its customized female luer-port screw cap. The customized cap consists of a standard female luer-port cap with a bent (by approximately 45°), 1.5 inch, 18 G, disposable needle installed through the cap and a small ball of Kimwipe inserted into the Luer port. It is important remove the cored-out polypropylene plug from the inside of the needle after installation. The cap was topped with a fritted 4-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003004).

The precipitation tube was prepared as follows: To a new, fritted 12-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003012) equipped with a PTFE-coated magnetic stir bar was added Celite® 545 filter aid (150 mg, not acid-washed, Acros Organics, Product # 349670025, Lot # A0287832) and 3-aminopropyl functionalized silica gel (250 mg, 40-63 μm, approximately 1 mmol/g NH_2, Sigma-Aldrich, Product # 364258, Lot # 79096HM). The cartridge was capped with its female Luer-port screw cap. To the

cartridge was added hexanes (5 mL, reagent grade) and the resulting suspension was swirled vigorously to mix the solids. The mixed suspension was allowed to settle for approximately 5 seconds and then the solvent was drained by forcing a plug of ambient air through the top of the cartridge by syringe. This process firmly embeds the stir bar in the solids to prevent stirring before the precipitation tube is utilized. This precipitation tube was wrapped with aluminum foil.

The silica gel chromatography column was prepared as follows: A silica gel chromatography column was freshly prepared from custom PTFE fittings using unfunctionalized silica gel. The cartridge was modeled after a 4-g cartridge (Luknova, Mansfield, MA, 02048, Part # FC003004), but was made of PTFE instead of polypropylene. To a clean, fritted column was added silica gel. This was done by vacuum aspiration through the bottom male Luer tip fitting. This process ensured tight, even packing of the silica gel plug. Excess silica gel was removed manually with a spatula and a 4-g frit (Luknova, Mansfield, MA, 02048, Part # FC003004) was placed on top of the silica plug. This doubly-fritted cartridge was capped with its female luer-port screw cap, using PTFE tape to ensure a tight seal.

The second reaction vessel was prepared as follows: To a non-flame-dried 7-mL glass vial equipped with a PTFE-coated magnetic stir bar was added palladium (II) acetate (1.2 mg, 0.0056 mmol, 5 mol%, ≥ 99.9%, Sigma-Aldrich, Product # 520764, Lot # 1000824996), 2-dicyclohexylphosphino-2',6'-dimethyoxy-1,1'-biphenyl (Sphos, 4.6 mg, 0.011 mmol, 10 mol%, 98%, Strem Chemicals, Product # 15-1143, Lot # 18526300), and anhydrous potassium phosphate (212 mg, 1 mmol, 9 equivs, 97%, Alfa Aesar, Product # L15168, Lot # L02U015). Potassium phosphate was freshly ground in a 100 °C mortar and pestle. This vial was sealed with a PTFE-lined septum screw cap. Through the septum was added a 1.5 inch, 20 G, disposable needle connected to a dry argon gas line. Then, through the septum was added a 1.5 inch, 20 G, disposable needle to act as a vent. The reaction vial was then flushed with dry argon for approximately 7 min. The vent needle and then the argon needle were removed from the septum.

The tubes, vessels and columns described above were used as follows: **See Figure 29** for reaction scheme. Both deprotection tubes (wrapped with aluminum foil) were securely installed on the machine. Tubes were installed by placing the tube's male Luer tip into the machine's appropriate female Luer port and were secured with a firm downward force and slight (less that one quarter turn) clockwise rotation. Both predrying tubes

(wrapped and topped with aluminum foil) were securely installed on the machine. Both drying tubes (wrapped in aluminum foil) were securely installed on the machine. Each drying tube was connected to the inert gas manifold by attaching a patch line to the machine's gas manifold and the tube's top Luer port. Patch lines are approximately 12-inch lengths of tubing with male luer-tip fittings on both ends. Both deoxygenating/concentrating tubes (wrapped in aluminum foil) were securely installed on the machine. Each deoxygenating/concentrating tube was connected to the inert gas manifold by attaching a vented patch line to the machine's gas manifold and the tube's top Luer port. Vented patch lines are approximately 12-inch lengths of tubing with a male luer-tip fitting on the machine-end and a Y-connector (one port connected to the line, one port connected to a male luer-tip fitting, and one port left open) on the tube-end. The first reaction tube was securely installed on the machine (in a heating block preheated to 40 °C) and connected to the inert gas manifold by attaching the reaction vent line to the tube's top Luer port. The reaction tube was then covered with aluminum foil and set to stirring at 600 rpm. The silica gel column was securely installed on the machine and connected to the purification module by attaching the eluent line to the column's top Luer port. The precipitation tube (wrapped in aluminum foil) was securely installed on the machine and connected to the purification module by placing the eluent line (fixed with a 1.5 inch, 18 G, disposable needle) through the tube's top Luer port).

The experiment's pre-assembled code was then loaded and executed to begin the automated sequence. The first aqueous MIDA boronate deprotection commenced immediately. After running the first deprotection (rt, 10 min), the machine quenched and worked up the resulting boronic acid solution and then dried, deoxygenated, and concentrated it. The machine then ran the first, slow addition, cross-coupling reaction (40 °C, 8 h total) and purified the resulting coupled product. The machine then ran the second aqueous MIDA boronate deprotection (rt, 10 min) and subsequently quenched, worked up, dried, deoxygenated, and concentrated the resulting boronic acid solution.

Approximately 5 minutes before the second cross-coupling began, the second reaction vessel was placed in an aluminum block (at room temperature) on a stir plate. An inert gas vent line (fixed with a 1.5 inch, 20 G, disposable needle) was connected, through the septum. The reaction tube was then covered with aluminum foil and set to stirring at 600 rpm. Separately, into a non-flame-dried 1.5-mL glass vial was added the aldehyde (16.6 mg, 0.11 mmol, 1 equiv). The vial was sealed with a septum screw cap and to this

was added 100 μL deoxygenated dry THF from a 100 μL, gas tight, fixed needle, glass syringe. The vial was manually gently agitated to dissolve the aldehyde and then was added to the reaction vial with the same syringe. The remaining residual aldehyde was quantitatively transferred to the reaction vial with 2 x 50 μL of deoxygenated dry THF using the same syringe. As the machine automatically deoxygenated the reaction addition line (fixed with a 1.5 inch, 22 G, disposable needle), it was connected to the reaction vessel, through the septum. The machine then ran the second, fast addition, cross-coupling reaction (rt, 3 h).

At the end of 3 hours, the reaction vial was removed from the machine and the crude reaction mixture was filtered through a 1-cm pad of Celite® packed in a pipette. The reaction vial was washed with 3 x 2 mL dry THF and these washings were filtered through the Celite® pad. The pad was then washed with 3 x 2 mL dry THF. The resulting clear dark yellow filtrate was concentrated *in vacuo* (rt, 80 Torr), azeotroped with 3 x 5 mL dichloromethane (rt, 80 Torr), and residual solvent was removed on high vacuum (30 min, 200 mTorr) to afford a dark yellow/orange sticky solid. This crude product was manually purified by silica gel flash chromatography to afford a mixture of all-*trans*-retinal:13-*cis*-retinal in a ratio of 1:0.55 in a combined total 30% yield.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The appended claims are not intended to claim all such embodiments and variations, and the full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

We claim:

1. A method of purifying a N-methyliminodiacetic acid (MIDA) boronate from a solution, comprising the steps of diluting the solution with hexane, thereby selectively precipitating the MIDA boronate; and isolating the precipitated MIDA boronate.

2. The method of claim 1, wherein the isolating comprises filtering the precipitated MIDA boronate.

3. The method of claim 1, further comprising the step of dissolving the precipitated MIDA boronate in tetrahydrofuran (THF).

4. The method of claim 1, wherein the solution comprising the MIDA boronate is a THF solution.

5. The method of claim 1, wherein the solution comprising the MIDA boronate is added dropwise to the hexane.

6. The method of claim 1, wherein the volume of hexane is between about two and about four times the volume of the solution.

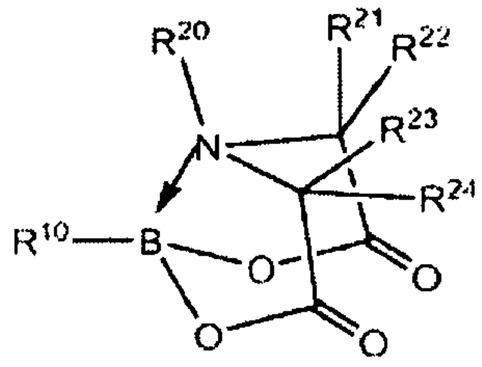
7. The method of claim 1, wherein the volume of hexane is about three times the volume of the solution.

8. The method of claim 1, wherein the solution comprising the MIDA boronate is a crude product mixture from a chemical reaction.

9. The method of claim 8, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

10. The method of claim 8, wherein the chemical reaction comprises the step of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

11. The method of claim 1, wherein the MIDA boronate is represented



by ; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of hydrogen and an organic group.

12. The method of claim 11, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

13. A method of purifying a N-methyliminodiacetic acid (MIDA) boronate from a solution, comprising the steps of passing the solution through a silica plug; passing a first liquid through the silica plug; and passing a second liquid through the silica plug, thereby eluting the MIDA boronate in the second liquid; wherein the first liquid contains diethyl ether or the polarity of the first liquid is less than or equal to about the polarity of a mixture of 98.5:1.5 (v/v) Et_2O:MeOH; and the polarity of the second liquid is greater than or equal to about the polarity of tetrahydrofuran (THF).

14. The method of claim 13, wherein the first liquid comprises diethyl ether.

15. The method of claim 13, wherein the first liquid is diethyl ether.

16. The method of claim 13, wherein the first liquid is a mixture of diethyl ether and methanol.

17. The method of claim 13, wherein first liquid is a mixture of diethyl ether and methanol; and the ratio of diethyl ether to methanol is 98.5:1.5 (v/v).

18. The method of claim 13, wherein the second liquid is THF, MeCN, ethyl acetate or acetone, or a solvent of similar polarity.

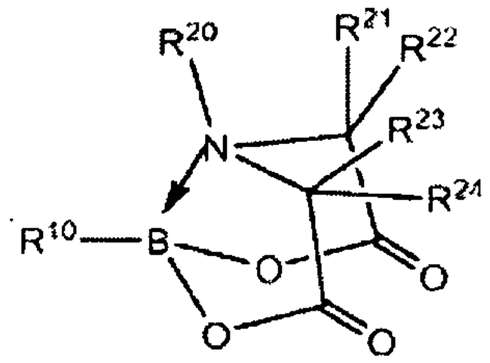
19. The method of claim 13, wherein the second liquid is THF, MeCN, ethyl acetate or acetone.

20. The method of claim 13, wherein the second liquid is THF.

21. The method of claim 13, wherein the solution is a crude product mixture from a chemical reaction.

22. The method of claim 21, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

23. The method of claim 21, wherein the chemical reaction comprises the steps of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

24. The method of claim 13, wherein the MIDA boronate is represented by  ; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24} independently are selected from the group consisting of hydrogen and an organic group.

25. The method of claim 24, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

26. A method of purifying a N-methyliminodiacetic acid (MIDA) boronate from a solution comprising the steps of diluting the solution with hexane, thereby selectively precipitating the MIDA boronate; passing the diluted solution through a silica plug, thereby depositing the precipitated MIDA boronate on the silica plug; passing a first liquid through the silica plug; and passing a second liquid through the silica plug, thereby eluting the MIDA boronate in the second liquid; wherein the first liquid contains diethyl ether or the polarity of the first liquid is less than or equal to about the polarity of a mixture of 98.5:1.5 (v/v) Et_2O:MeOH; and the polarity of the second liquid is greater than or equal to about the polarity of tetrahydrofuran (THF).

27. The method of claim 26, wherein the first liquid comprises diethyl ether.

28. The method of claim 26, wherein the first liquid is diethyl ether.

29. The method of claim 26, wherein the first liquid is a mixture of diethyl ether and methanol.

30. The method of claim 26, wherein first liquid is a mixture of diethyl ether and methanol; and the ratio of diethyl ether to methanol is 98.5:1.5 (v/v).

31. The method of claim 26, wherein the second liquid is THF, MeCN, ethyl acetate or acetone, or a solvent of similar or greater polarity.

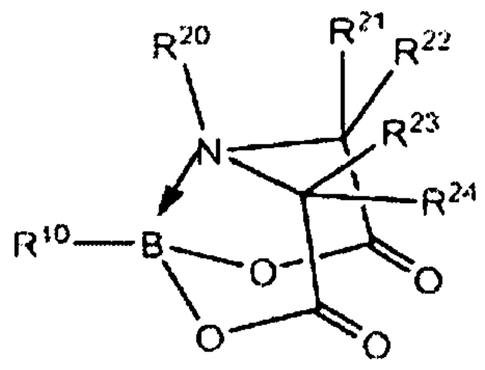
32. The method of claim 26, wherein the second liquid is THF, MeCN, ethyl acetate or acetone.

33. The method of claim 26, wherein the second liquid is THF.

34. The method of claim 26, wherein the solution is a crude product mixture from a chemical reaction.

35. The method of claim 34, wherein the chemical reaction is selected from the group consisting of a Suzuki-Miyaura coupling, an oxidation, a Swern oxidation, a "Jones reagents" oxidation, a reduction, an Evans' aldol reaction, an HWE olefination, a Takai olefination, an alcohol silylation, a desilylation, a *p*-methoxybenzylation, an iodination, a Negishi cross-coupling, a Heck coupling, a Miyaura borylation, a Stille coupling, and a Sonogashira coupling.

36. The method of claim 34, wherein the chemical reaction comprises the steps of contacting a MIDA boronate with a reagent, wherein the MIDA boronate comprises a boron having an sp^3 hybridization, a MIDA protecting group bonded to the boron, and an organic group bonded to the boron through a boron-carbon bond; the organic group is chemically transformed, and the boron is not chemically transformed.

37. The method of claim 26, wherein the MIDA boronate is represented by 

; R^{10} represents an organic group; B represents boron having sp^3 hybridization; R^{20} is methyl; and R^{21}, R^{22}, R^{23} and R^{24}

independently are selected from the group consisting of hydrogen and an organic group.

38. The method of claim 37, wherein R^{21}, R^{22}, R^{23} and R^{24} are hydrogen.

39. A method of deprotecting a N-methyliminodiacetic acid (MIDA) boronate comprising the step of contacting a solution comprising the MIDA boronate and a solvent with a solid-supported ammonium hydroxide reagent, thereby deprotecting the MIDA boronate and forming a boronic acid and a MIDA.

40. The method of claim 39, wherein the solvent comprises THF.

41. The method of claim 39, wherein the solid-supported ammonium hydroxide reagent binds the MIDA.

42. The method of claim 39, further comprising the steps of removing the solvent by filtration, leaving the boronic acid and MIDA ligand trapped inside the solid-supported ammonium hydroxide reagent; and adding additional solvent.

43. The method of claim 42, wherein the additional solvent is THF.

44. The method of claim 39, further comprising washing the solid-supported ammonium hydroxide reagent with an organic solution comprising an organic solvent and an acid in a quantity greater than that needed to neutralize the solid-supported ammonium hydroxide reagent, thereby eluting the boronic acid.

45. The method of claim 44, wherein the organic solvent is THF.

46. The method of claim 45, wherein the acid is acetic acid.

47. The method of claim 46, further comprising treating the eluted boronic acid with base to neutralize the acetic acid.

48. The method of claim 47, wherein the base is potassium carbonate.

49. The method of claim 39, further comprising washing the solid-supported ammonium hydroxide reagent with a 1,4-dioxane solution comprising hydrochloric acid, thereby eluting the boronic acid.

50. The method of claim 39, wherein the solid-supported ammonium hydroxide reagent is a strong base anion exchange resin.

51. The method of claim 39, wherein the solid-supported ammonium hydroxide reagent is a strong base, type 1, anionic, macroreticular polymeric resin based on crosslinked styrene divinylbenzene copolymer containing quaternary ammonium groups.

52. A method of deprotecting an N-methyliminodiacetic acid (MIDA) boronate, comprising the step of contacting a solution comprising the MIDA boronate and a solvent with an aqueous solution of NaOH, thereby deprotecting the MIDA boronate and forming a boronic acid and free MIDA ligand.

53. The method of claim 52, wherein the solvent comprises tetrahydrofuran (THF).

54. The method of claim 52, further comprising the steps of adding diethyl ether, thereby generating a biphasic mixture comprising an organic phase comprising the deprotected MIDA boronate and an aqueous phase; and isolating the organic phase comprising the boronic acid and free MIDA ligand from the aqueous phase.

55. The method of claim 54, further comprising the step of contacting the organic phase with one or more drying agents selected from the group consisting of magnesium sulfate, diatomaceous earth, and molecular sieves, thereby drying the organic phase comprising the boronic acid and free MIDA ligand.

56. The method of claim 55, further comprising deoxygenating the dried organic phase comprising the boronic acid and free MIDA ligand.

57. The method of claim 56, wherein the deoxygenating comprises bubbling dry oxygen-free gas through the organic phase comprising the boronic acid and free MIDA ligand.

58. The method of claim 57, wherein the oxygen-free gas is argon.

59. An automated small molecule synthesizer comprising:

(a) a deprotection module, in fluid communication with

(b) a drying and degassing module, in fluid communication with

(c) a reaction module, in fluid communication with

(d) a purification module;

(e) at least one pump which can move liquid from one module to another; and

(f) a computer equipped with software;

wherein all of the modules are under control of the computer.

60. The automated small molecule synthesizer of claim 59, wherein the deprotection module comprises solid-supported ammonium hydroxide reagent.

61. The automated small molecule synthesizer of claim 59, wherein the deprotection module is an aqueous deprotection module.

62. The automated small molecule synthesizer of claim 59, comprising a plurality of deprotection modules; a plurality of drying and degassing modules; a plurality of reaction modules; a plurality of purification modules; at least one pump which can move liquid from one module to another; and a computer equipped with software; wherein all of the modules are under the control of the computer.

Application number / numéro de demande: 2842410

Figures:

Pages: 2, 6—9,

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant étre balayés
(Commander les documents originaux dans la section de préparation des dossiers au 10ème étage)

Figure 1

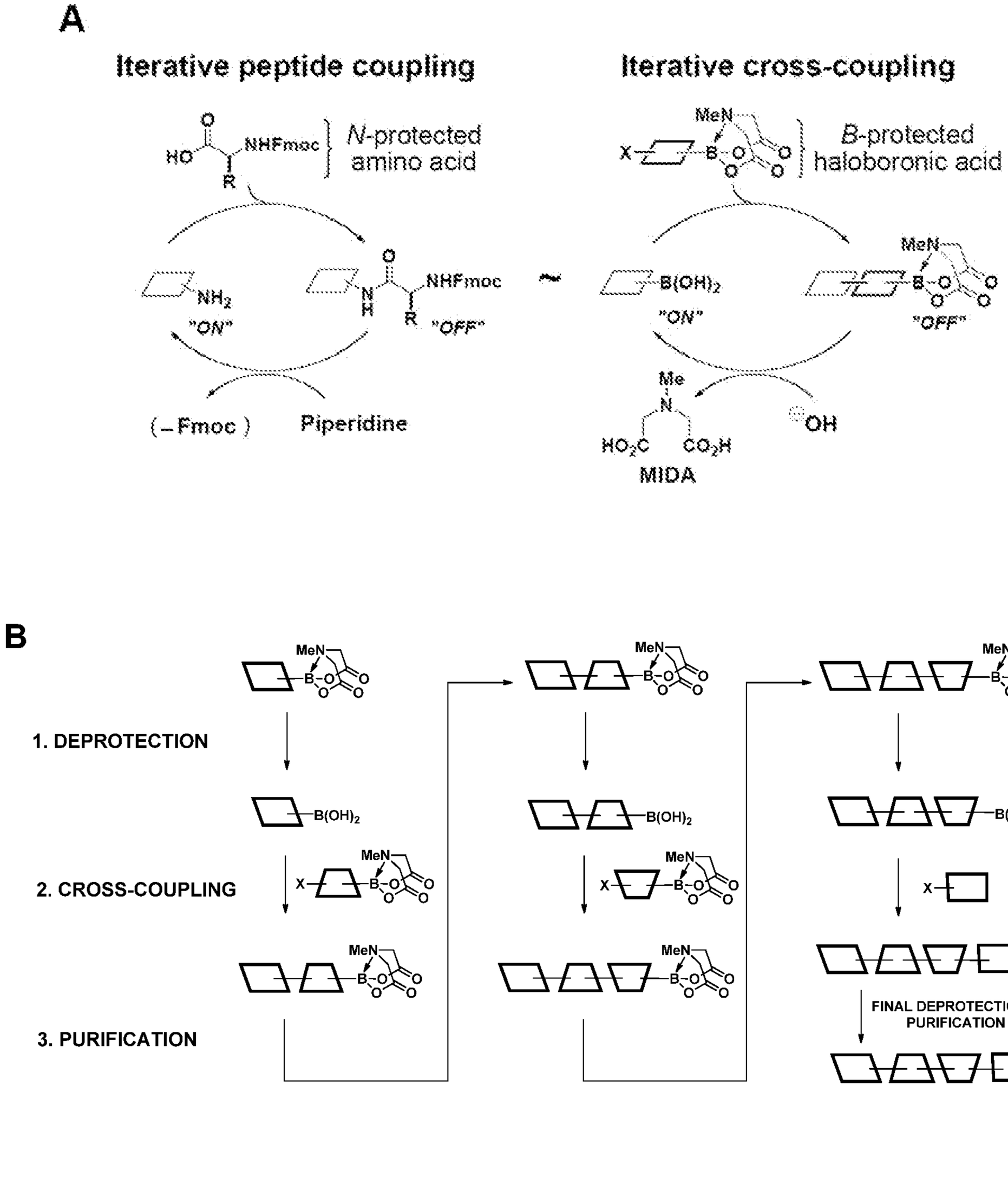

A
Iterative peptide coupling
Iterative cross-coupling
N-protected amino acid
B-protected haloboronic acid
NHFmoc
"ON"
"OFF"
(–Fmoc)
Piperidine
B(OH)2
MIDA
B
1. DEPROTECTION
2. CROSS-COUPLING
3. PURIFICATION
FINAL DEPROTECTIONS/ PURIFICATION

Figure 2

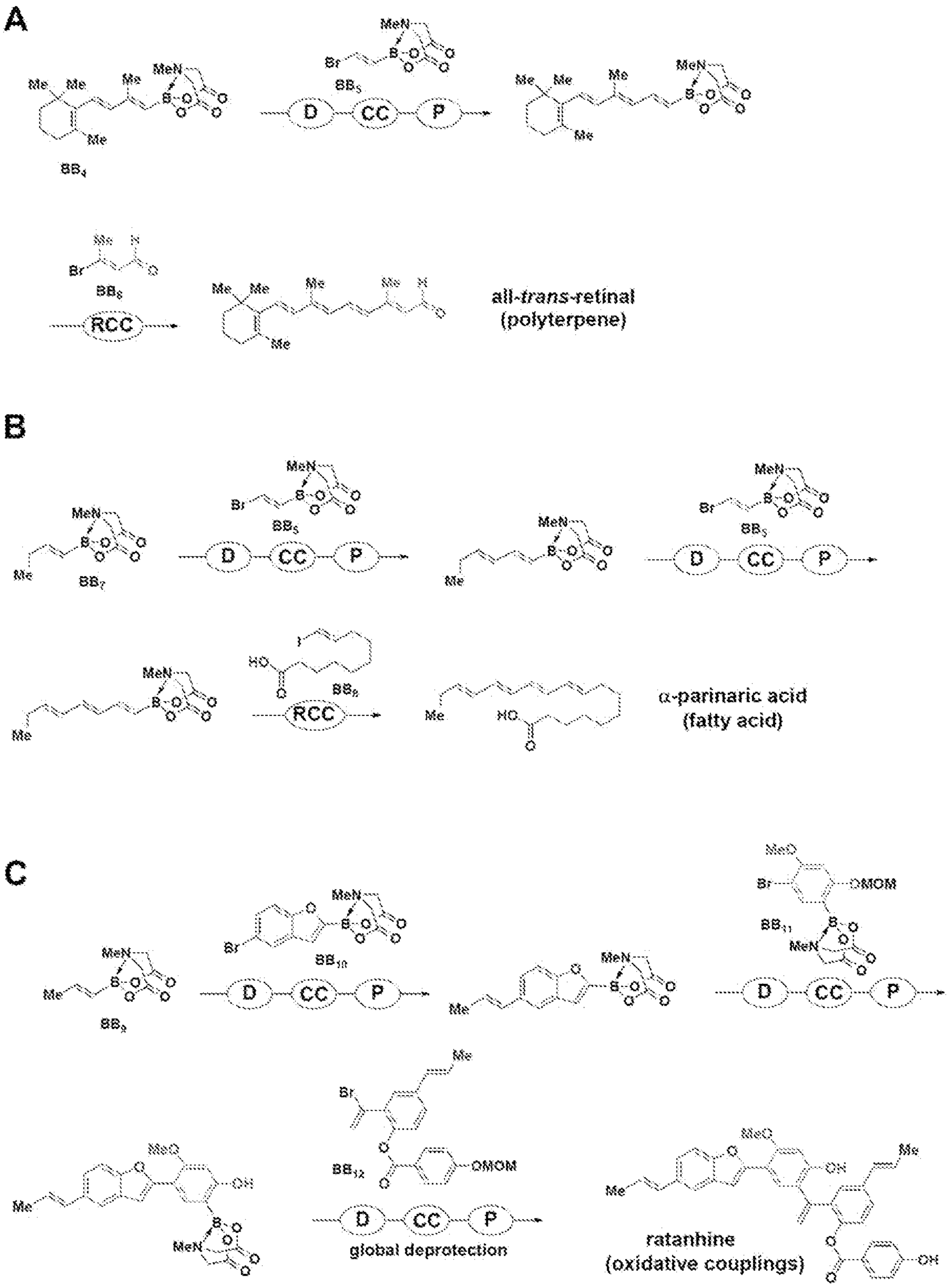

A
MeN
Me
Me
Me
Me
Me
Br
BB_5
D
CC
P
BB_8
RCC
all-*trans*-retinal
(polyterpene)
B
BB_7
BB_9
α-parinaric acid
(fatty acid)
C
BB_6
BB_{10}
MeO
OMOM
BB_{11}
BB_{12}
global deprotection
ratanhine
(oxidative couplings)

Figure 2 (continued)

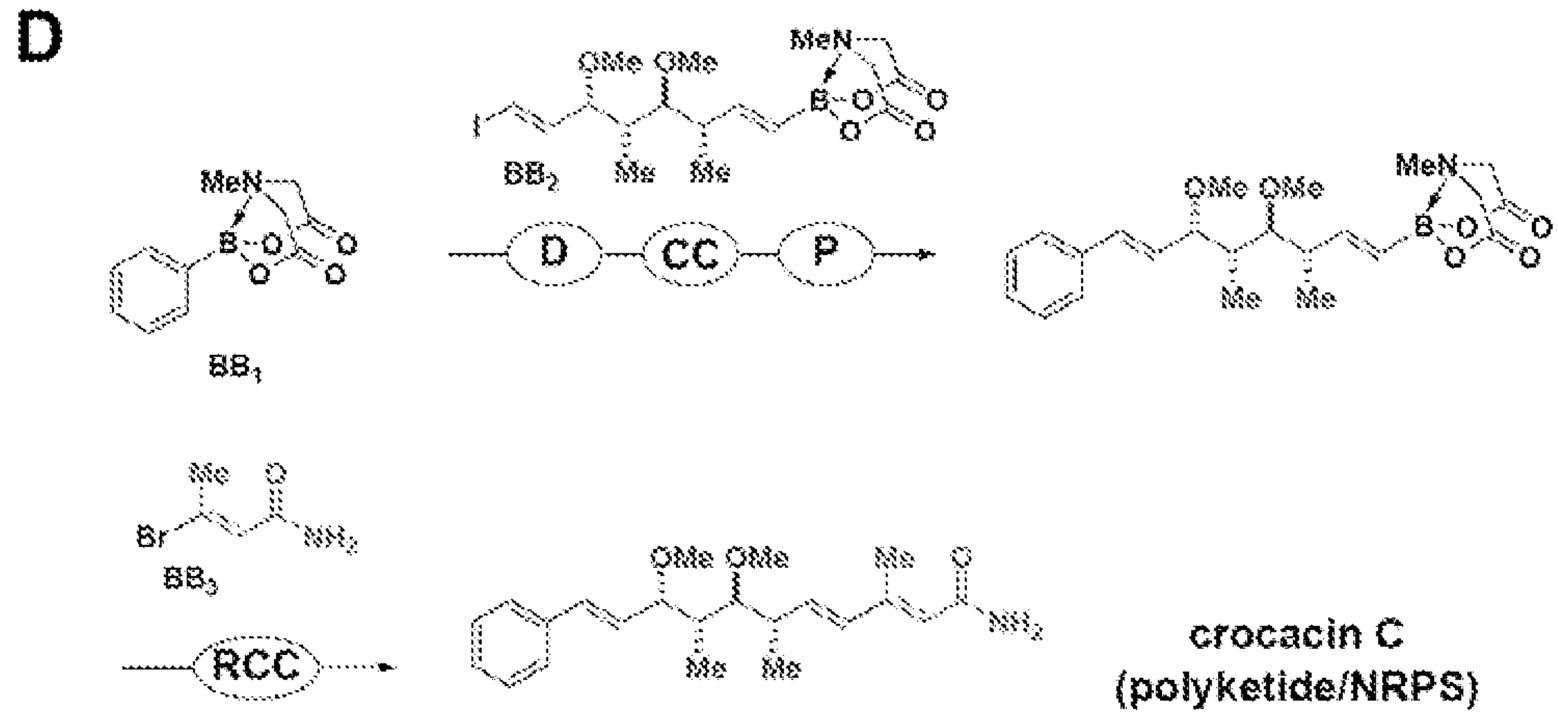


Figure 3

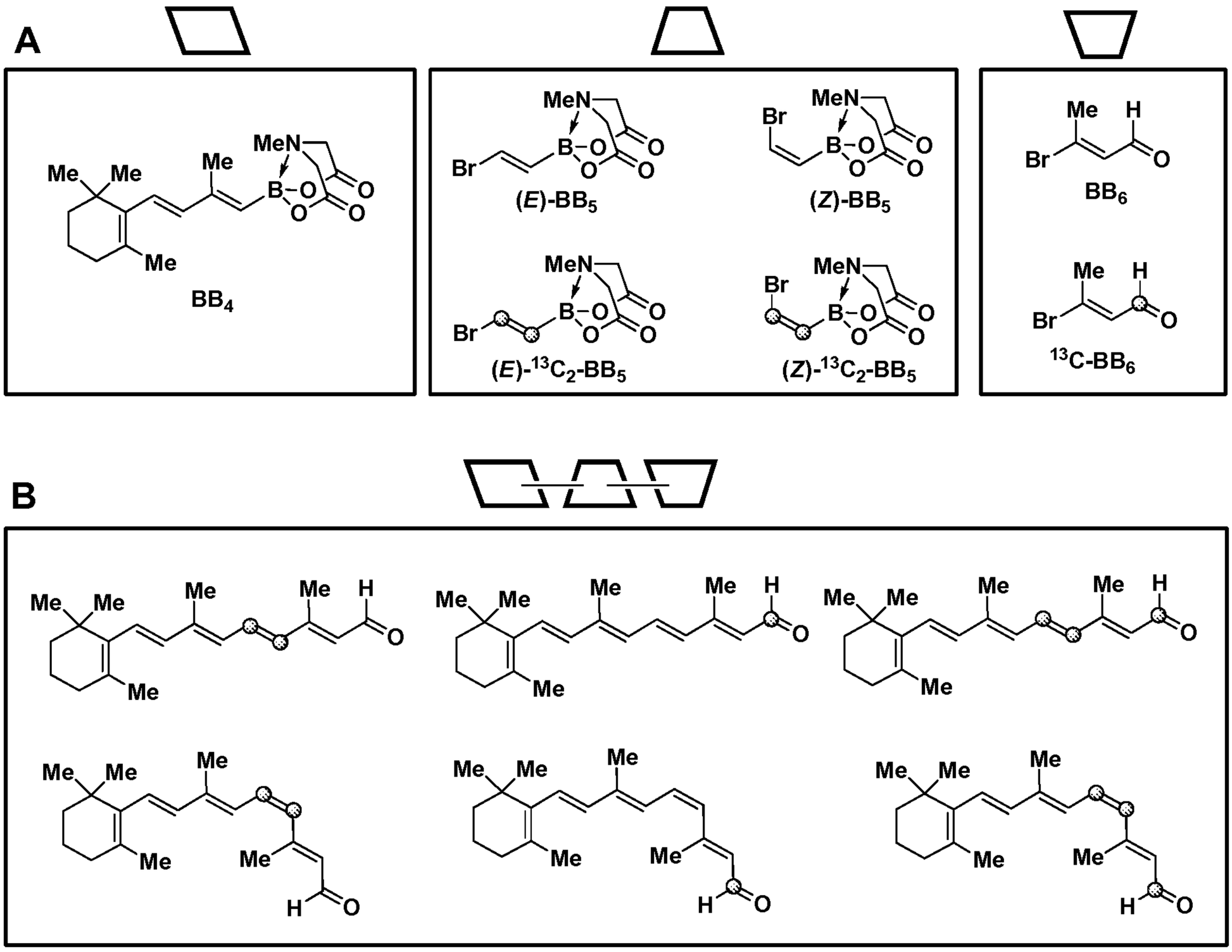


Figure 4

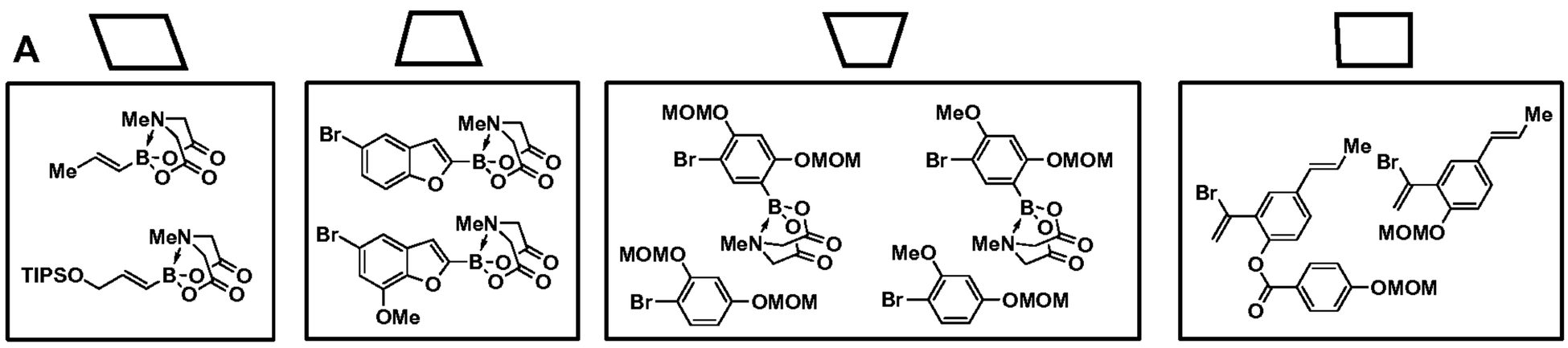

A
MeN
B
O
Me
TIPSO
Br
OMe
MOMO
OMOM
MeO
B
neolignan natural products *to be* prepared via automated ICC
HO
OH
109194-71-0
91432-06-3
143001-80-3
ratanhine
neolignan natural product analogs *to be* prepared via automated ICC

Figure 9

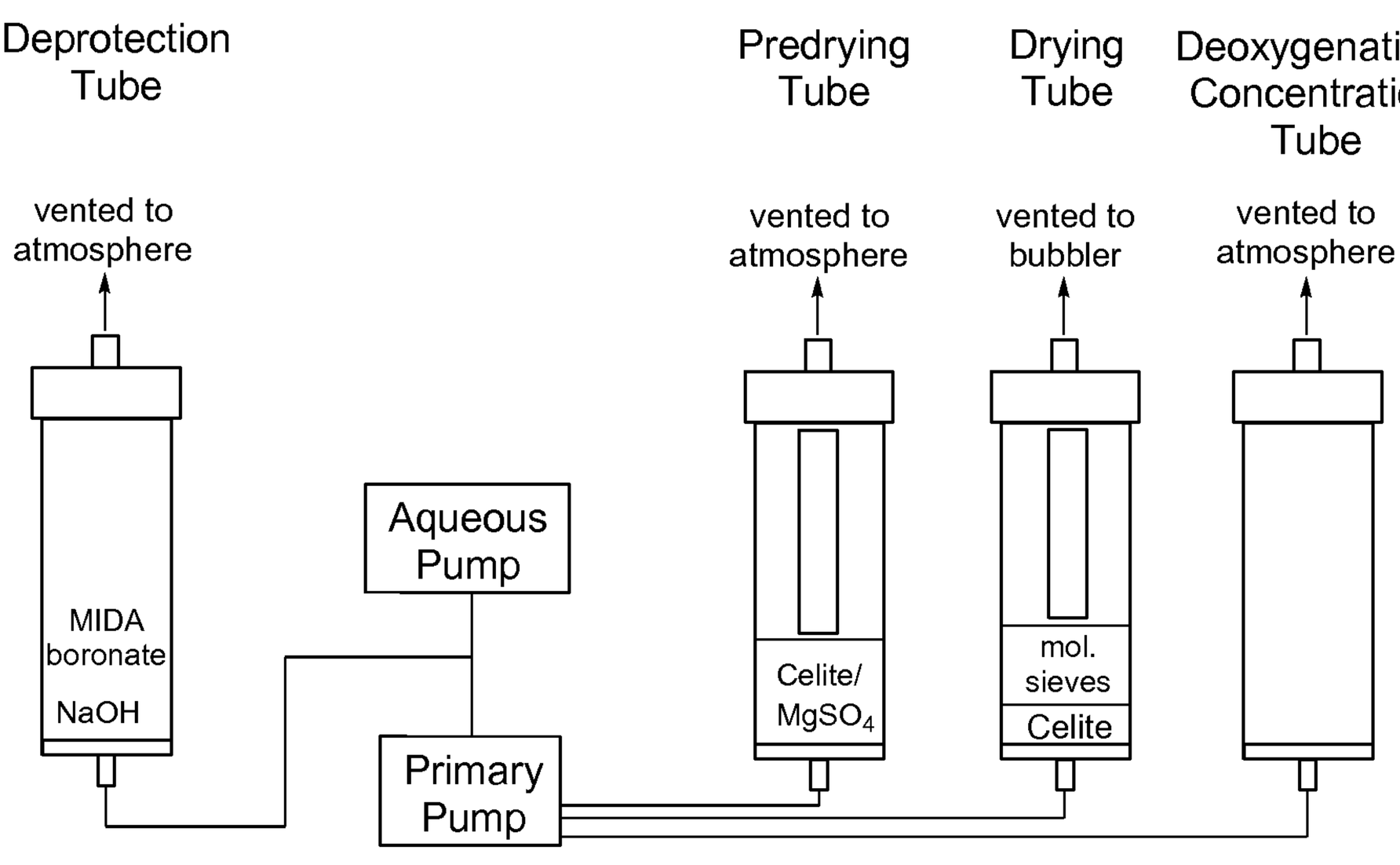

Deprotection Tube
vented to atmosphere
MIDA boronate
NaOH
Aqueous Pump
Primary Pump
Predrying Tube
vented to atmosphere
Celite/ $MgSO_4$
Drying Tube
vented to bubbler
mol. sieves
Celite
Deoxygenating/ Concentration Tube
vented to atmosphere

Figure 10

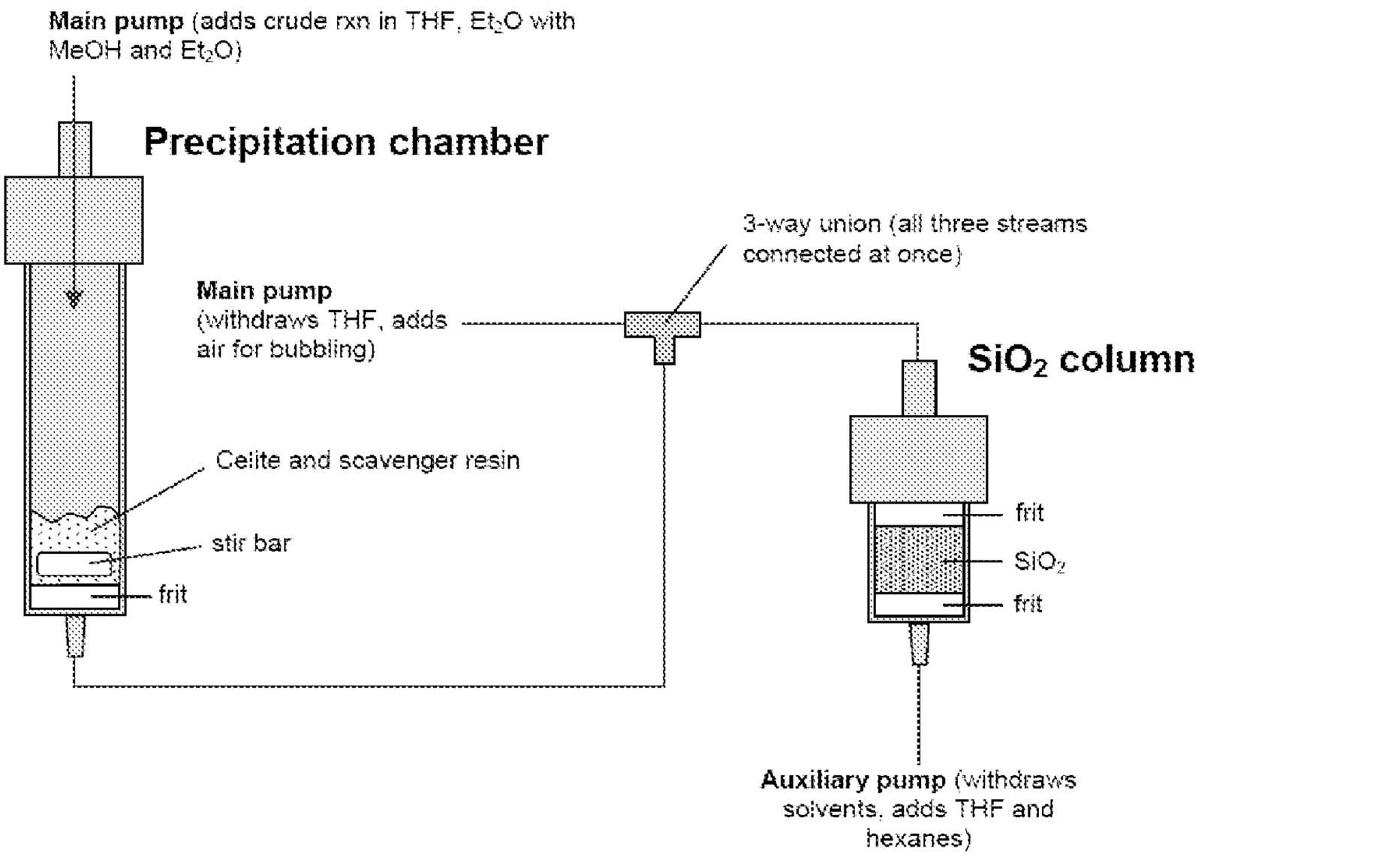
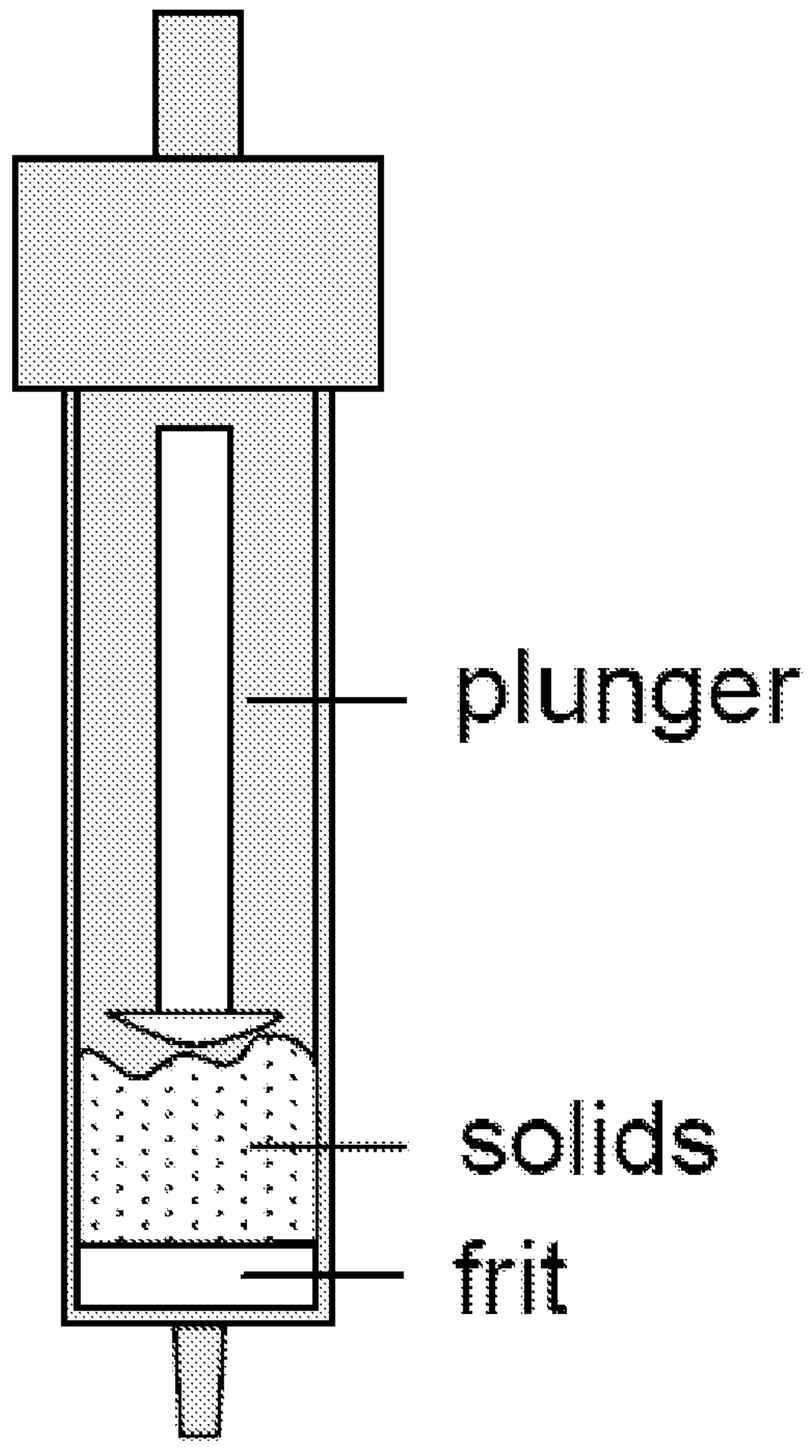

Main pump (adds crude rxn in THF, Et_2O with MeOH and Et_2O)
Precipitation chamber
3-way union (all three streams connected at once)
Main pump (withdraws THF, adds air for bubbling)
SiO_2 column
Celite and scavenger resin
stir bar
frit
frit
SiO_2
frit
Auxiliary pump (withdraws solvents, adds THF and hexanes)

Figure 11



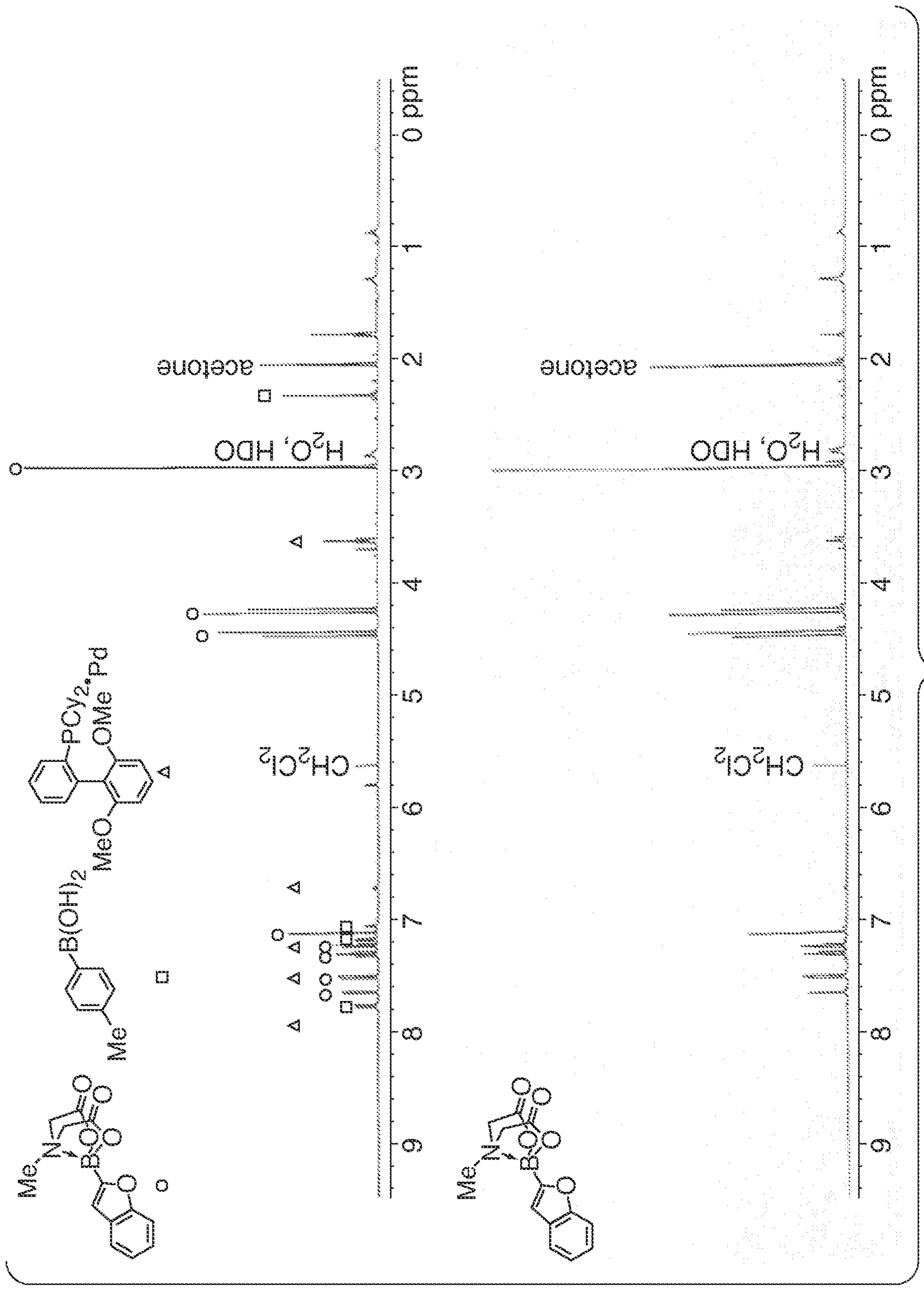

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
$B(OH)_2$
MeO
PCy_2•Pd
OMe
Me
Me
N
B
O

Figure 12

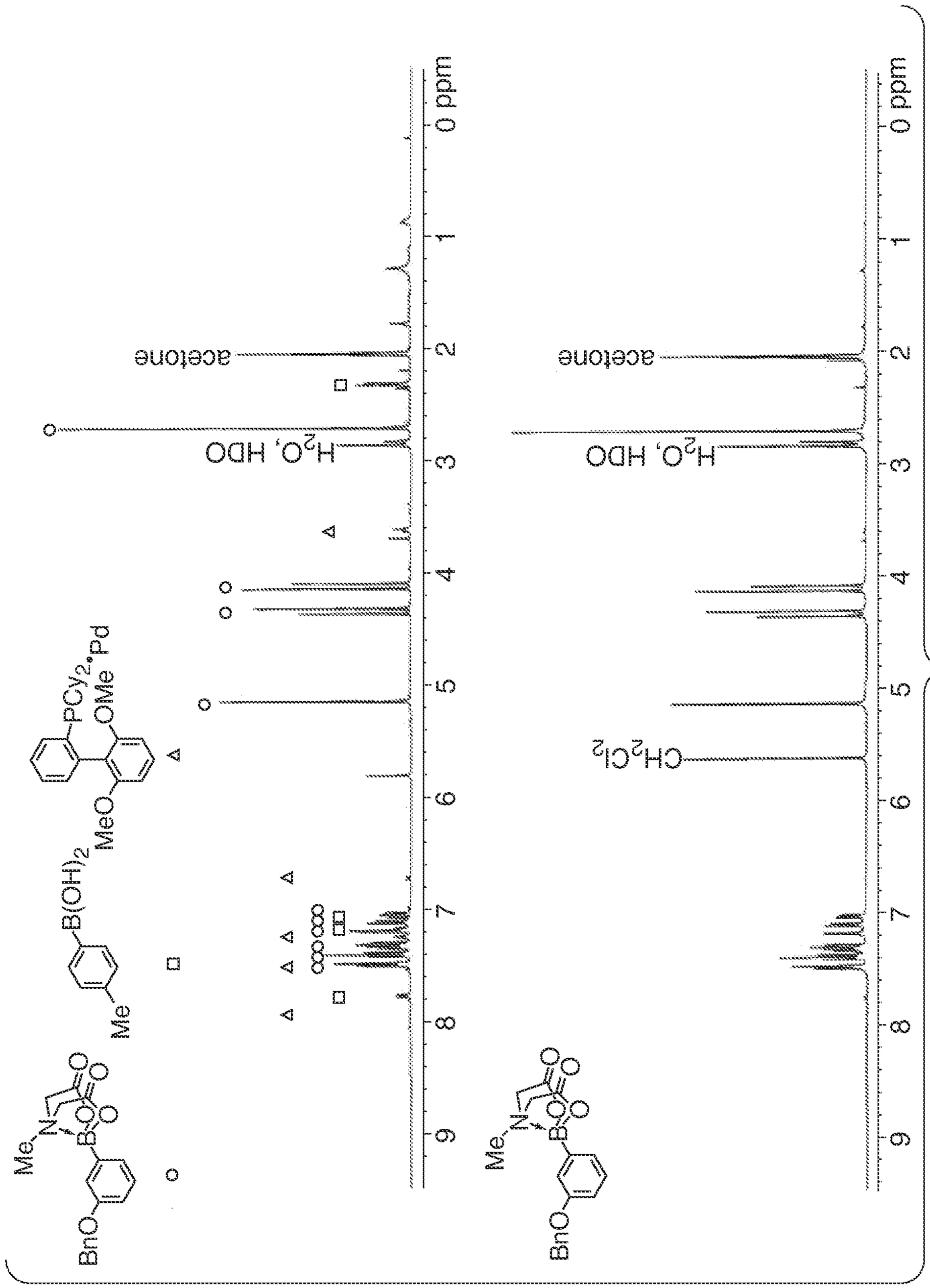

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
Me
$B(OH)_2$
MeO
PCy_2
OMe
Pd
BnO

Figure 13

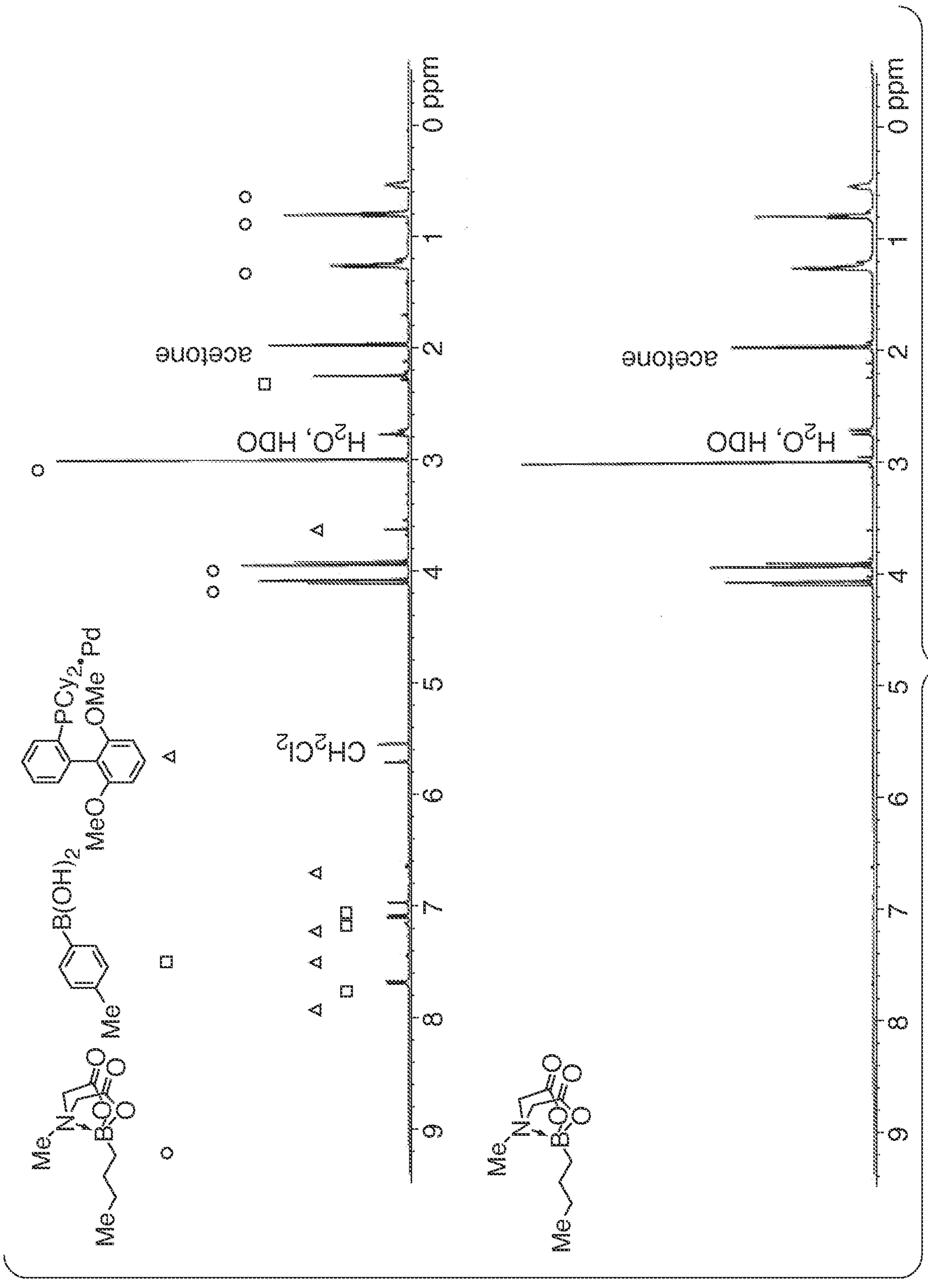

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
$B(OH)_2$
MeO
PCy_2
OMe
Pd
Me
N
B
O

Figure 14

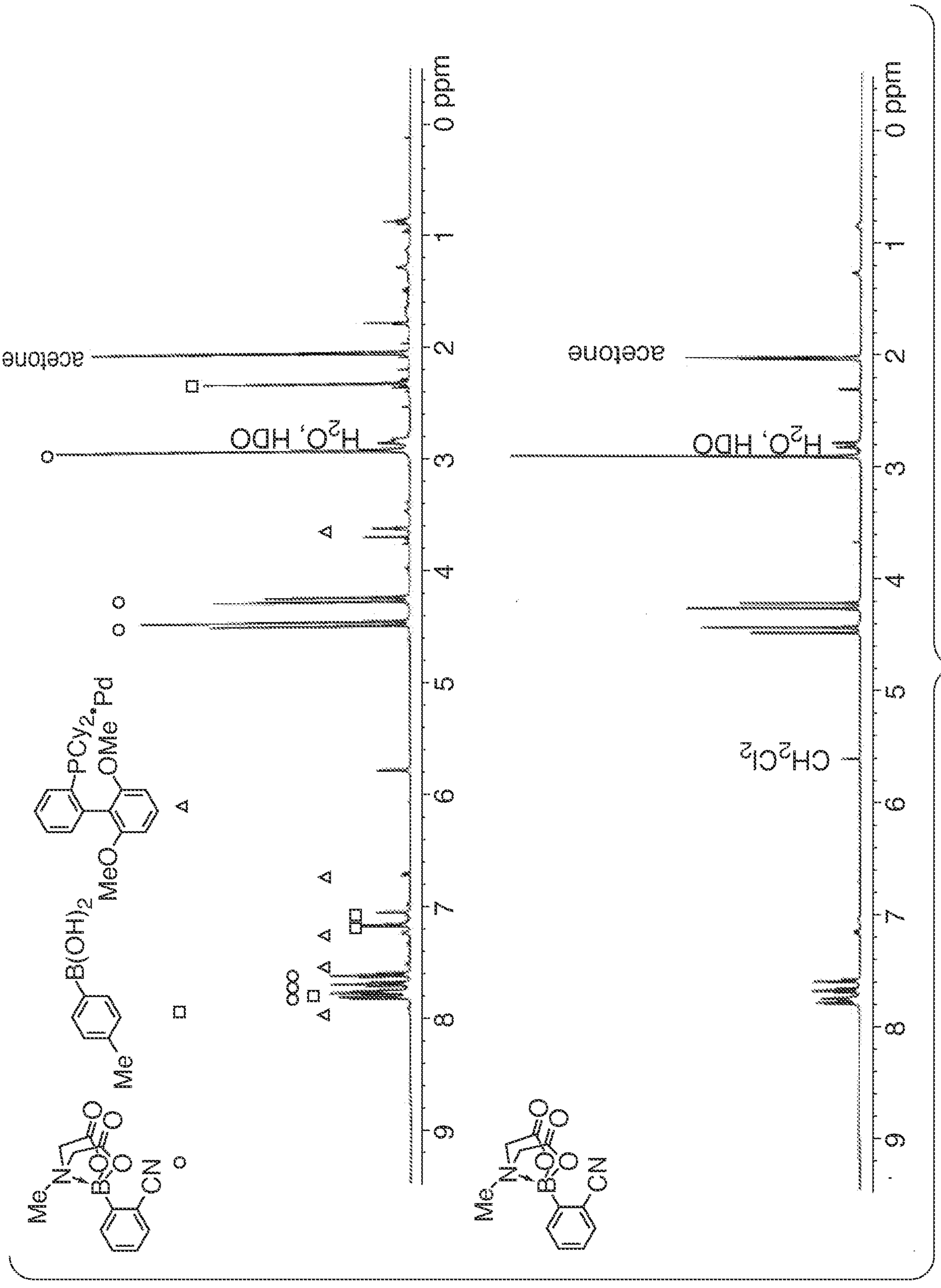


Figure 15

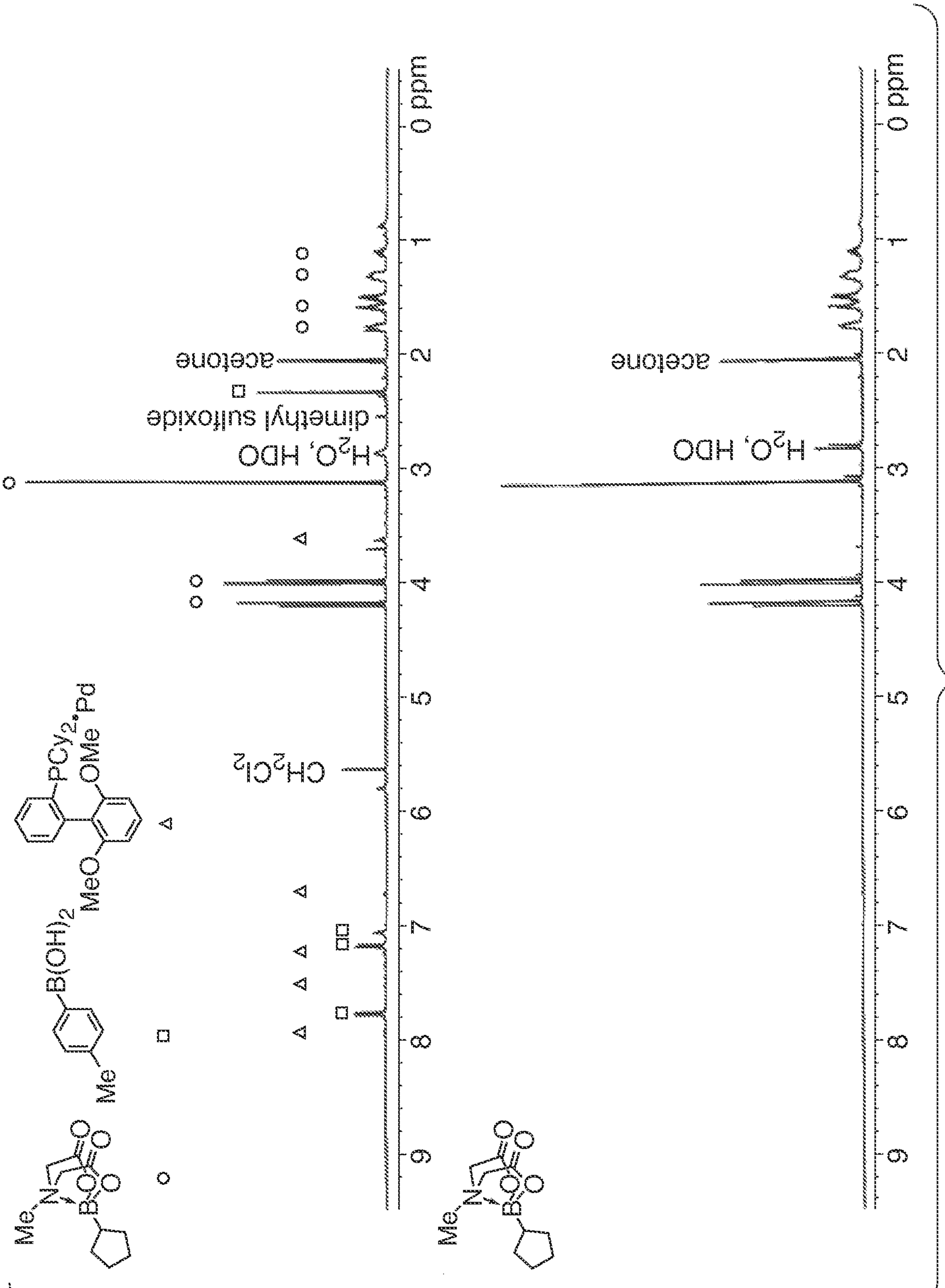

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
dimethyl sulfoxide
CH_2Cl_2
$B(OH)_2$
MeO
PCy_2
OMe
Pd
Me
Me
N
B
O

Figure 16

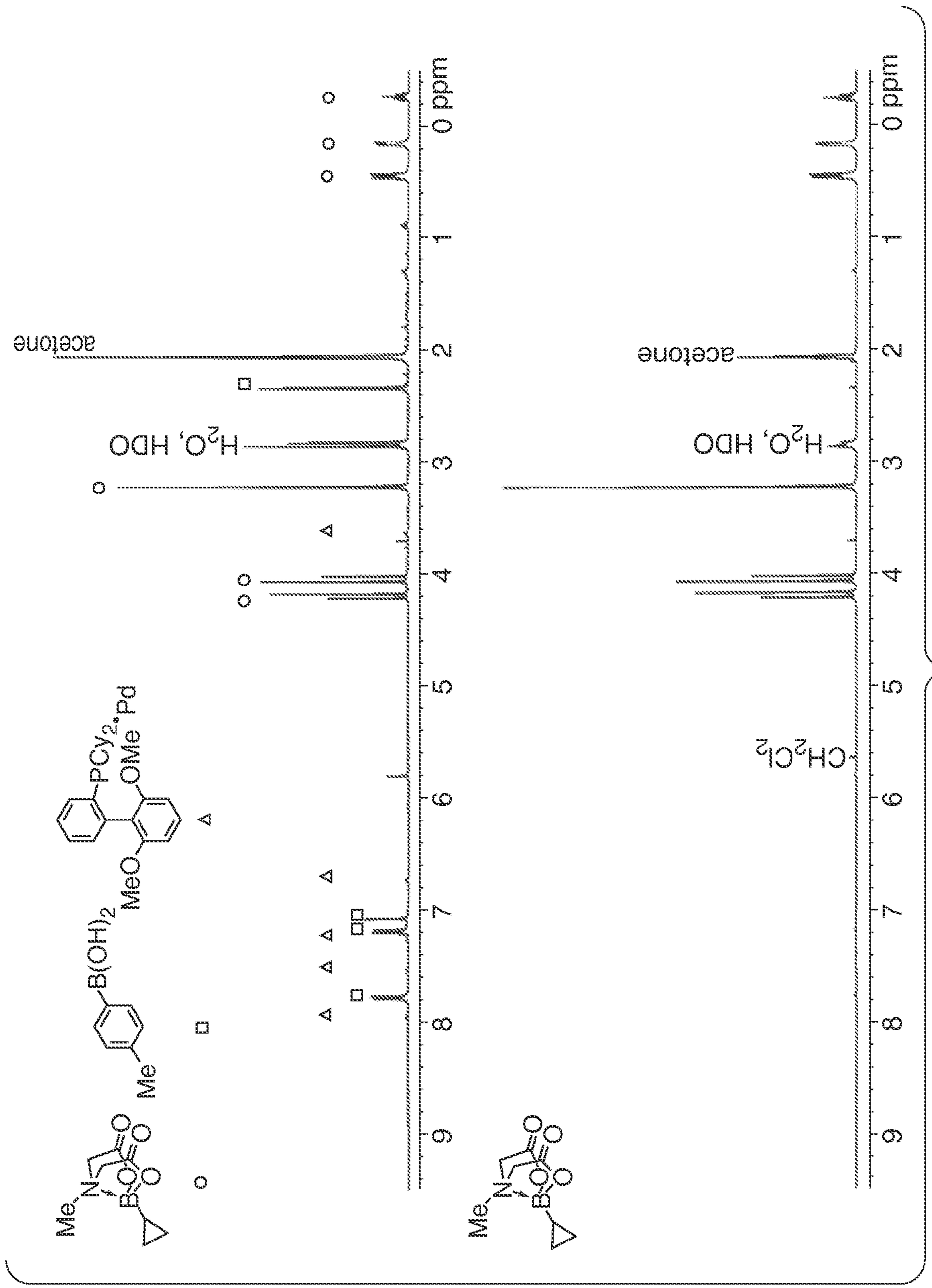

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
PCy_2
OMe
Pd
MeO
$B(OH)_2$
Me
N
B
O

Figure 17

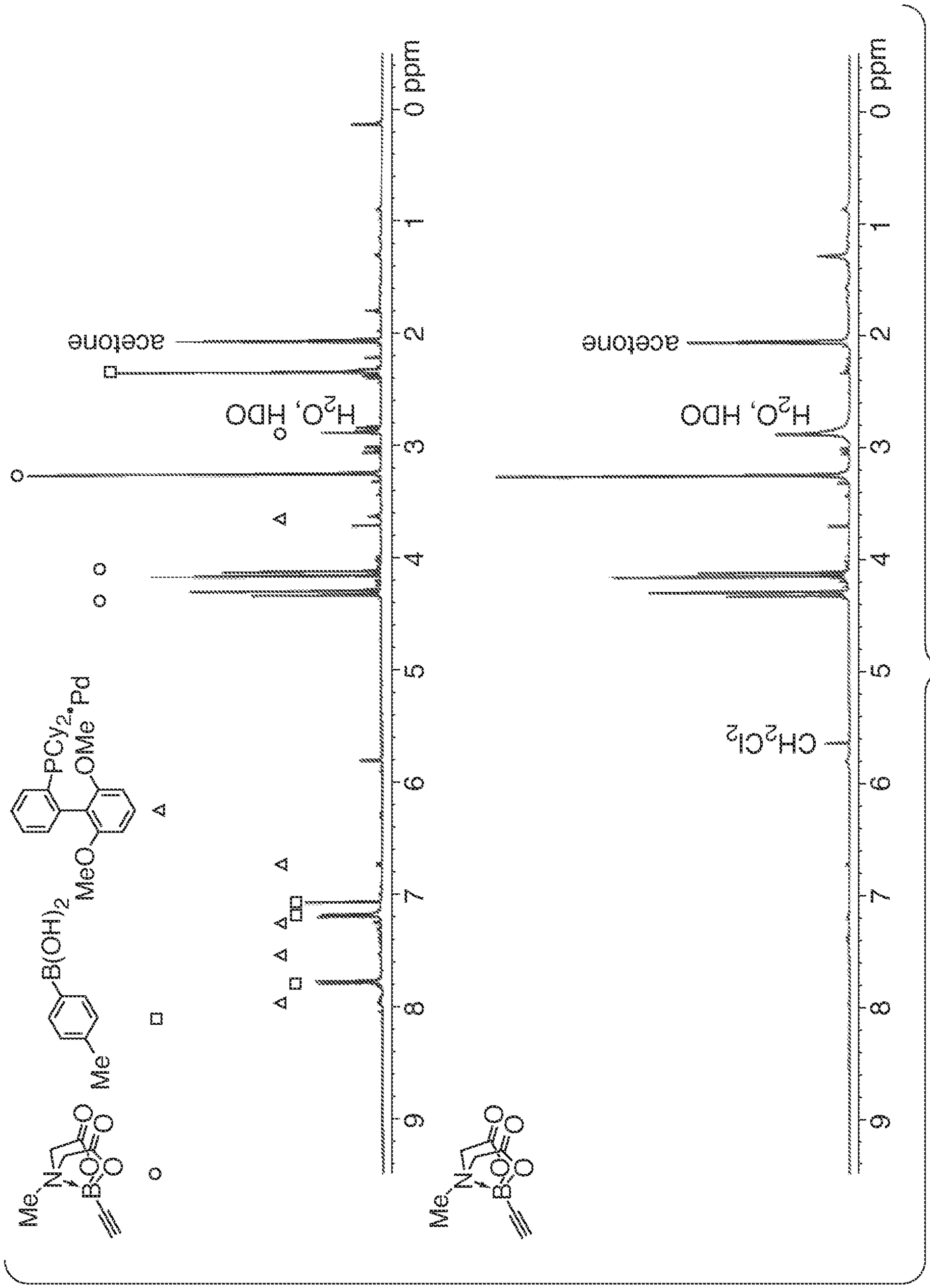

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H2O, HDO
CH2Cl2
Me
B(OH)2
MeO
PCy2
OMe
Pd
Me
O
O
O
O
B
O
N
Me

Figure 18

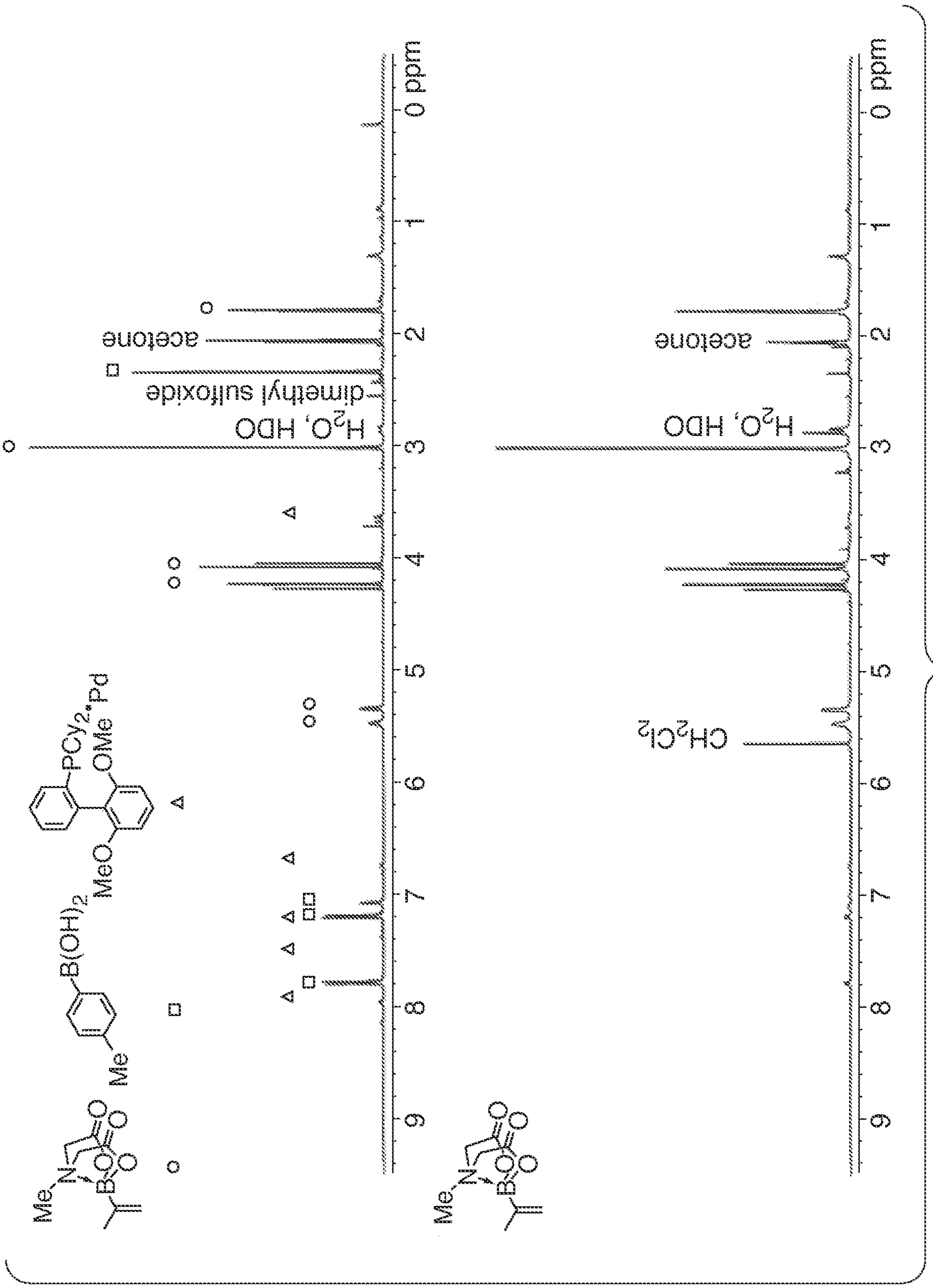

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H2O, HDO
CH2Cl2
0 ppm
1
2
3
4
5
6
7
8
9
acetone
H2O, HDO
dimethyl sulfoxide
CH2Cl2
B(OH)2
PCy2
OMe
Pd
MeO
Me
N
B
O

Figure 19

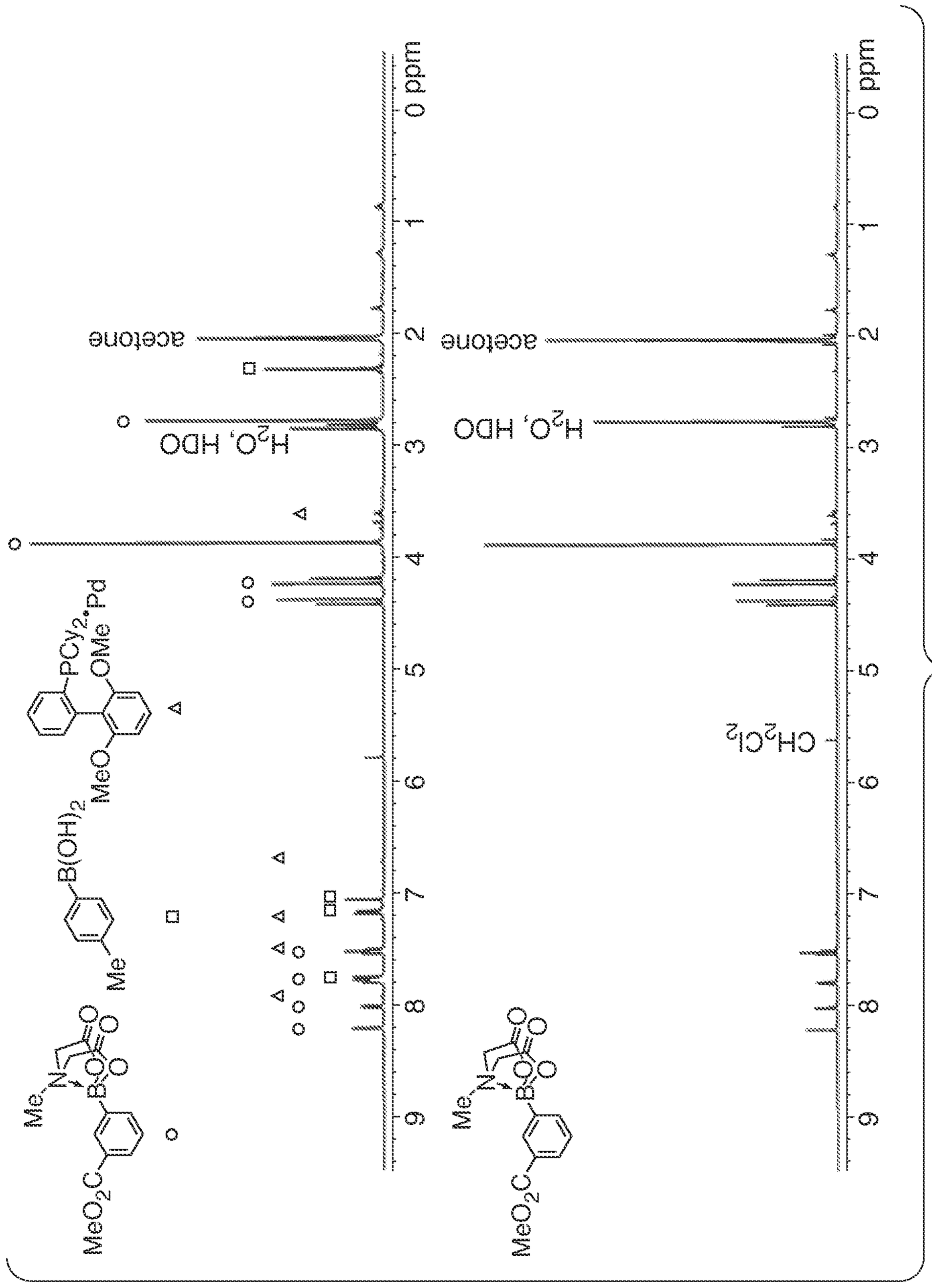

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
$B(OH)_2$
Me
MeO
PCy_2
OMe
Pd
MeO_2C
N
Me
B
O

Figure 20

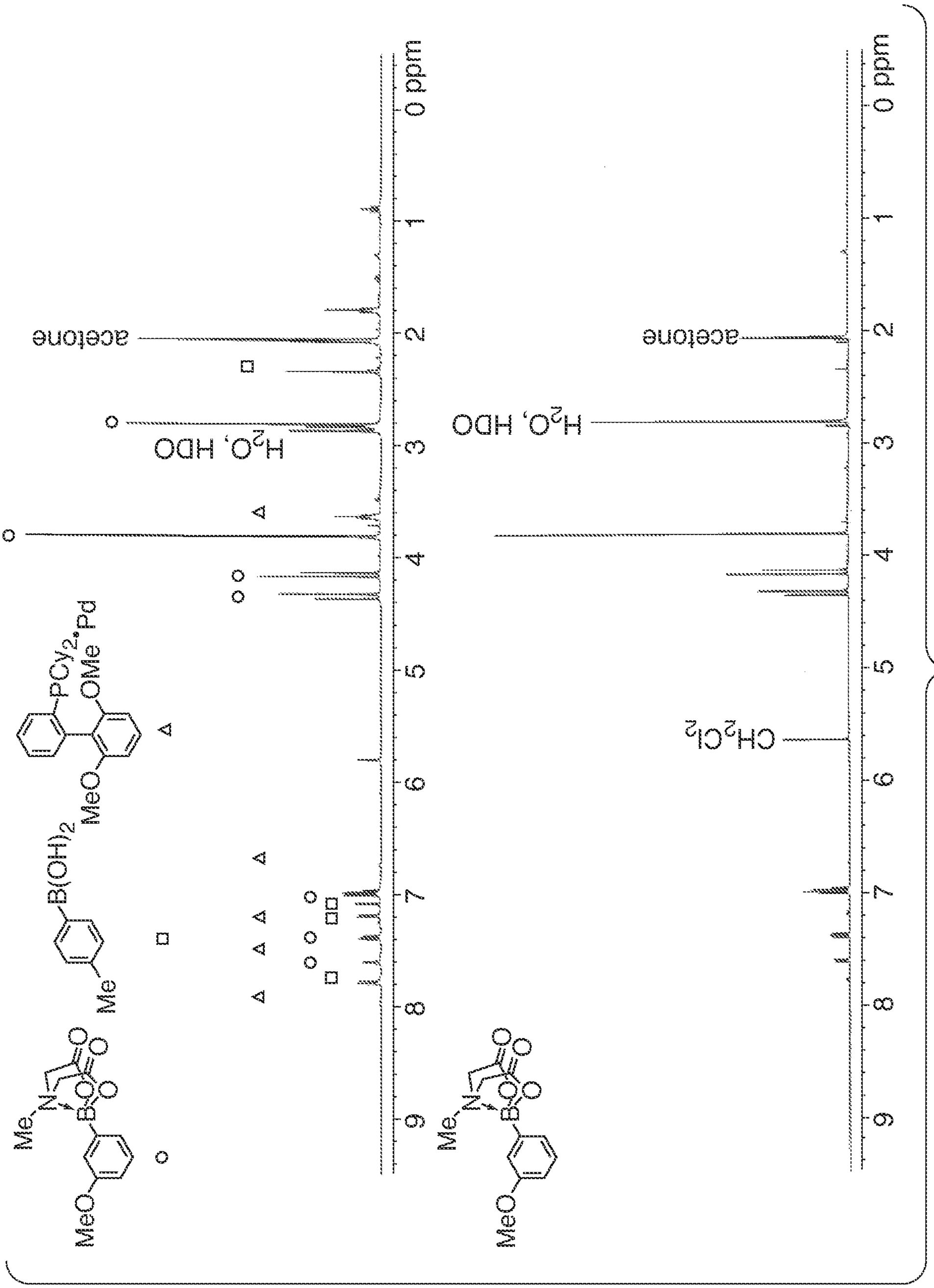

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
PCy_2
OMe
MeO
$B(OH)_2$
Me
Me
N
B
O
MeO

Figure 21

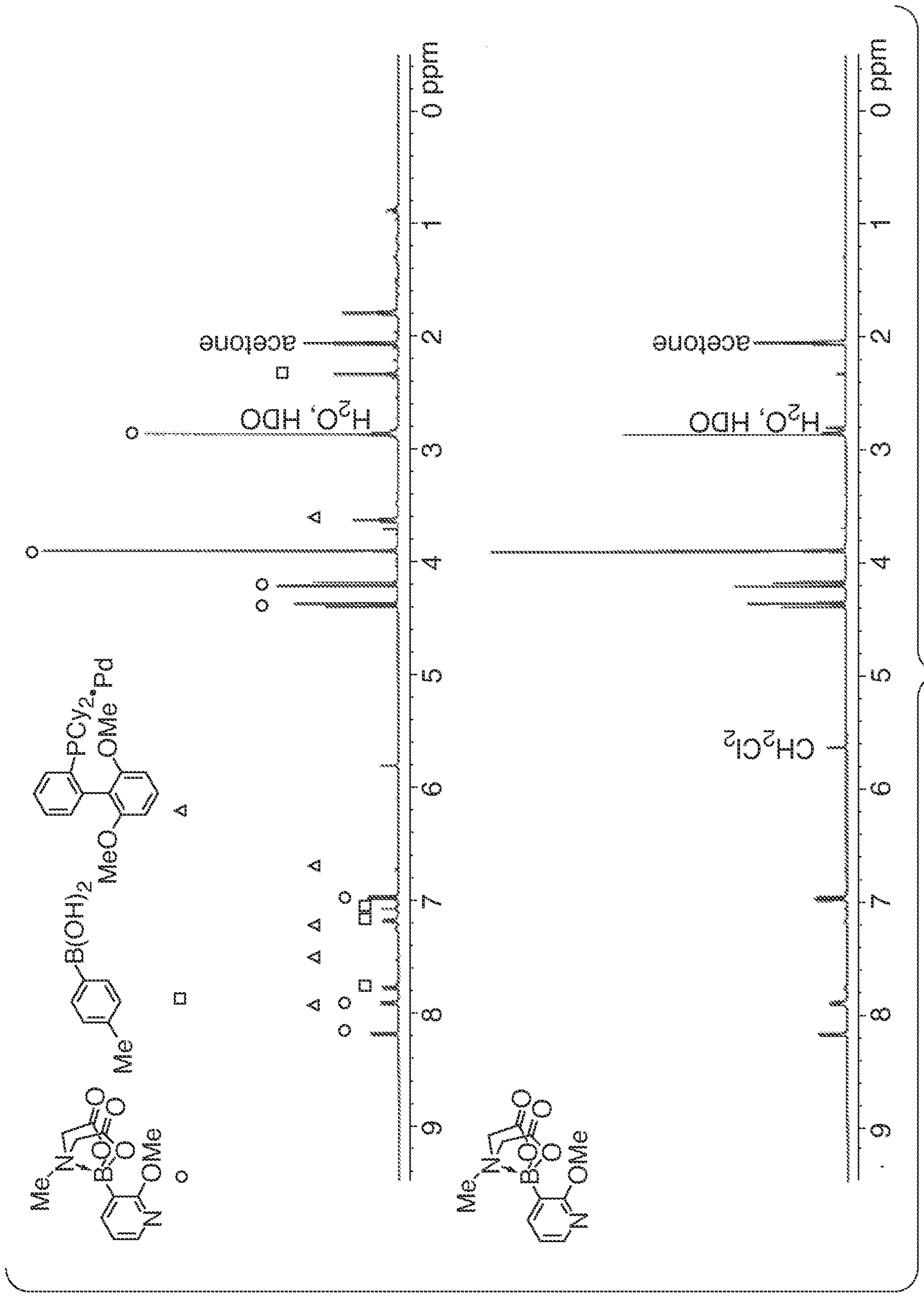

0 ppm
1
2
3
4
5
6
7
8
9
H_2O, HDO
acetone
CH_2Cl_2
$B(OH)_2$
MeO
PCy_2
OMe
Pd
Me
N
B
O
OMe

Figure 22

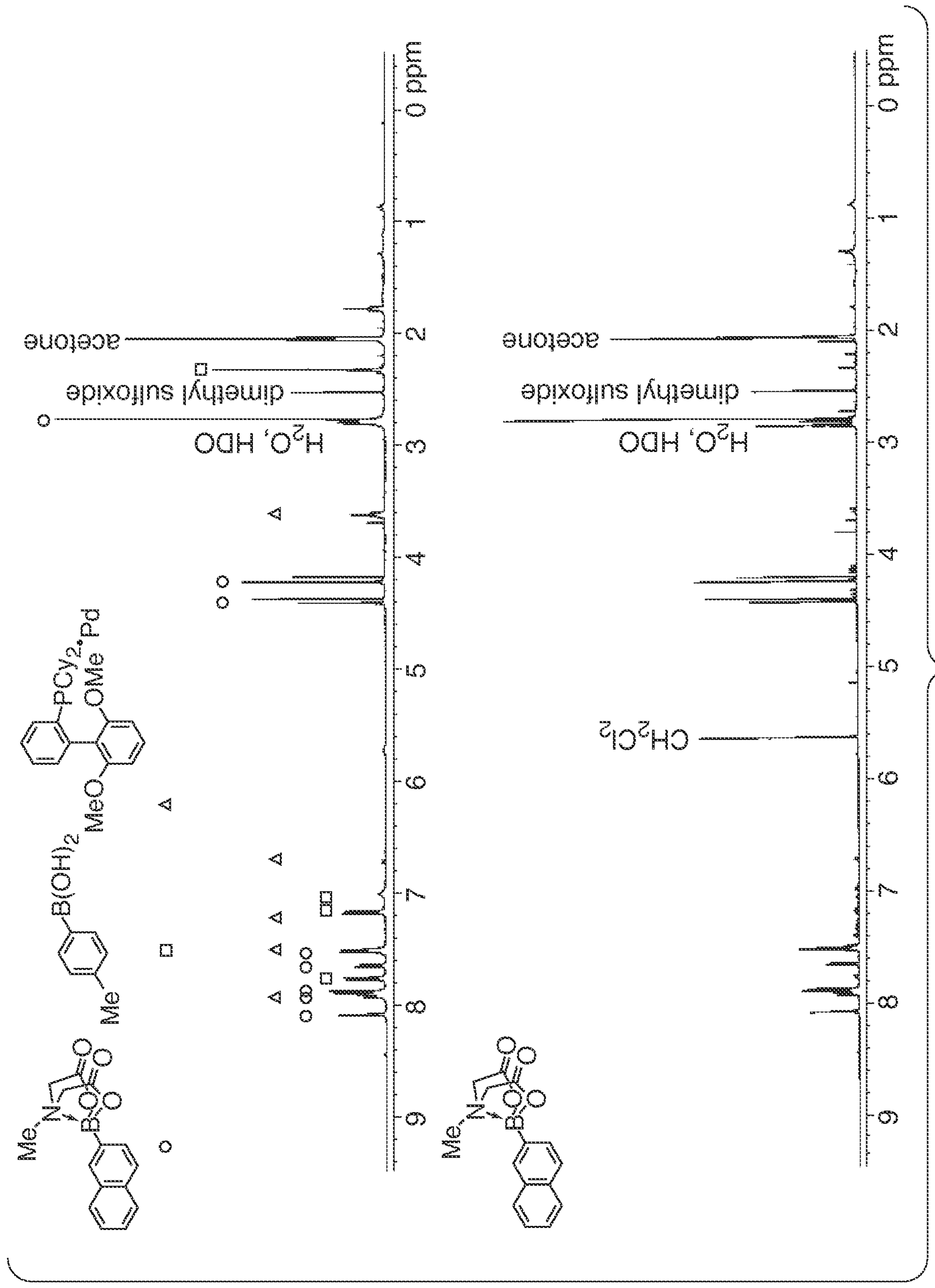

ppm
0
1
2
3
4
5
6
7
8
9
H_2O, HDO
dimethyl sulfoxide
acetone
CH_2Cl_2
$B(OH)_2$
Me
MeO
PCy_2
OMe
Pd
N
Me
O
O
O
O
B
O

Figure 23

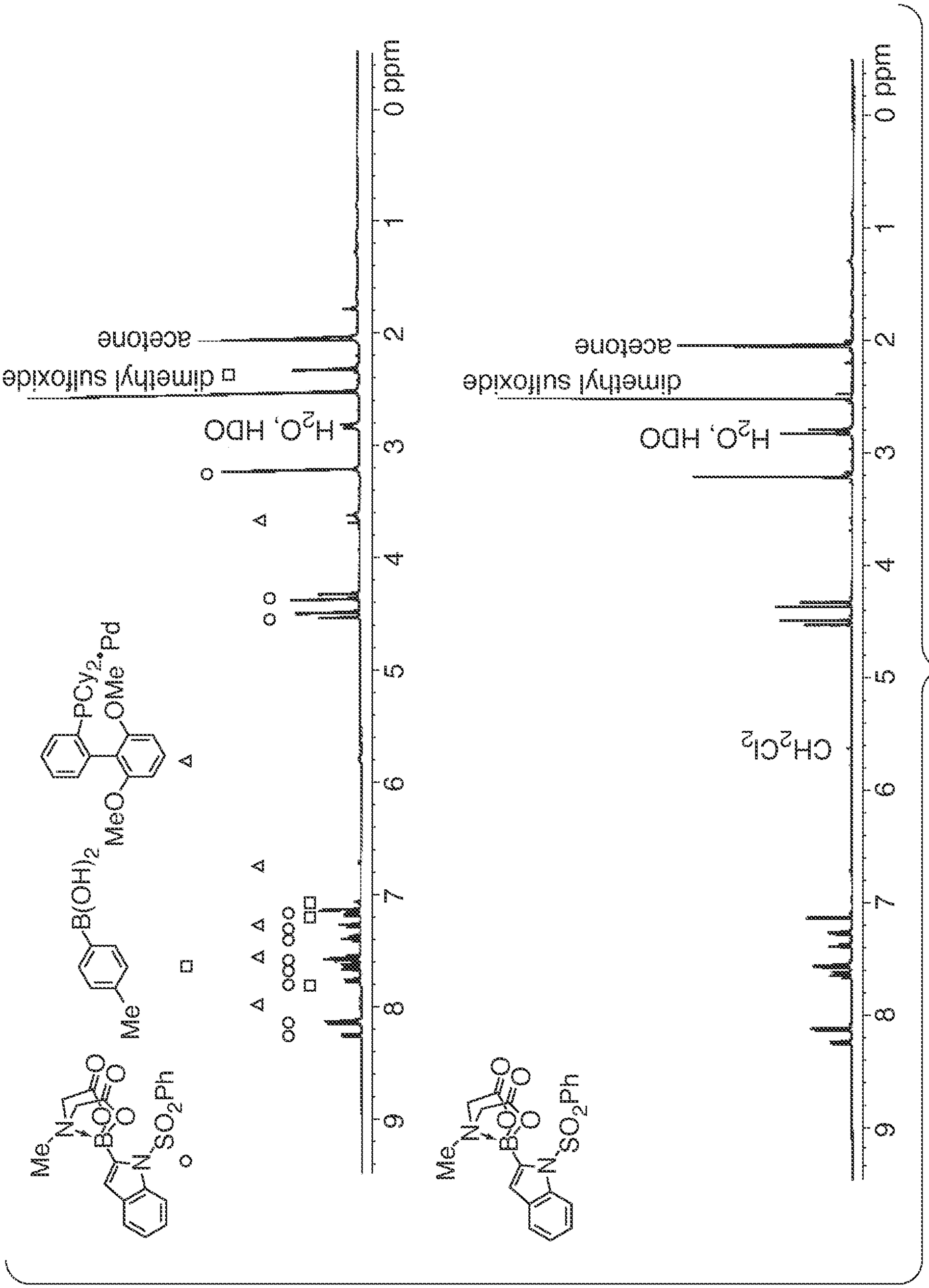

dimethyl sulfoxide
acetone
H_2O, HDO
CH_2Cl_2
ppm
dimethyl sulfoxide
acetone
H_2O, HDO
CH_2Cl_2
ppm
$B(OH)_2$
Me
MeO
PCy_2
OMe
Pd
Me
SO_2Ph
Me
SO_2Ph

Figure 24

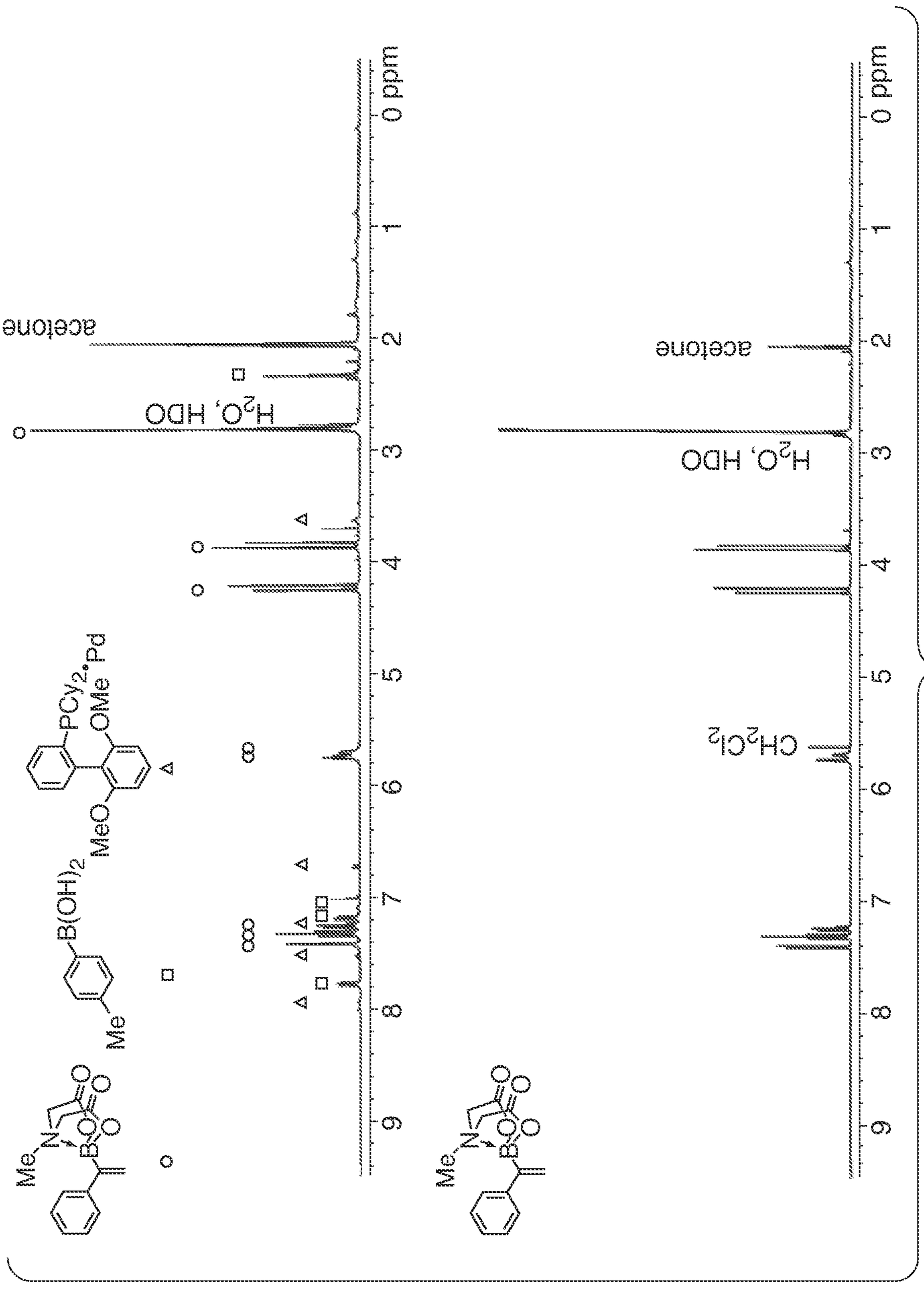

ppm 0
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
PCy_2
OMe
Pd
MeO
$B(OH)_2$
Me
N
B
O
Me

Figure 25

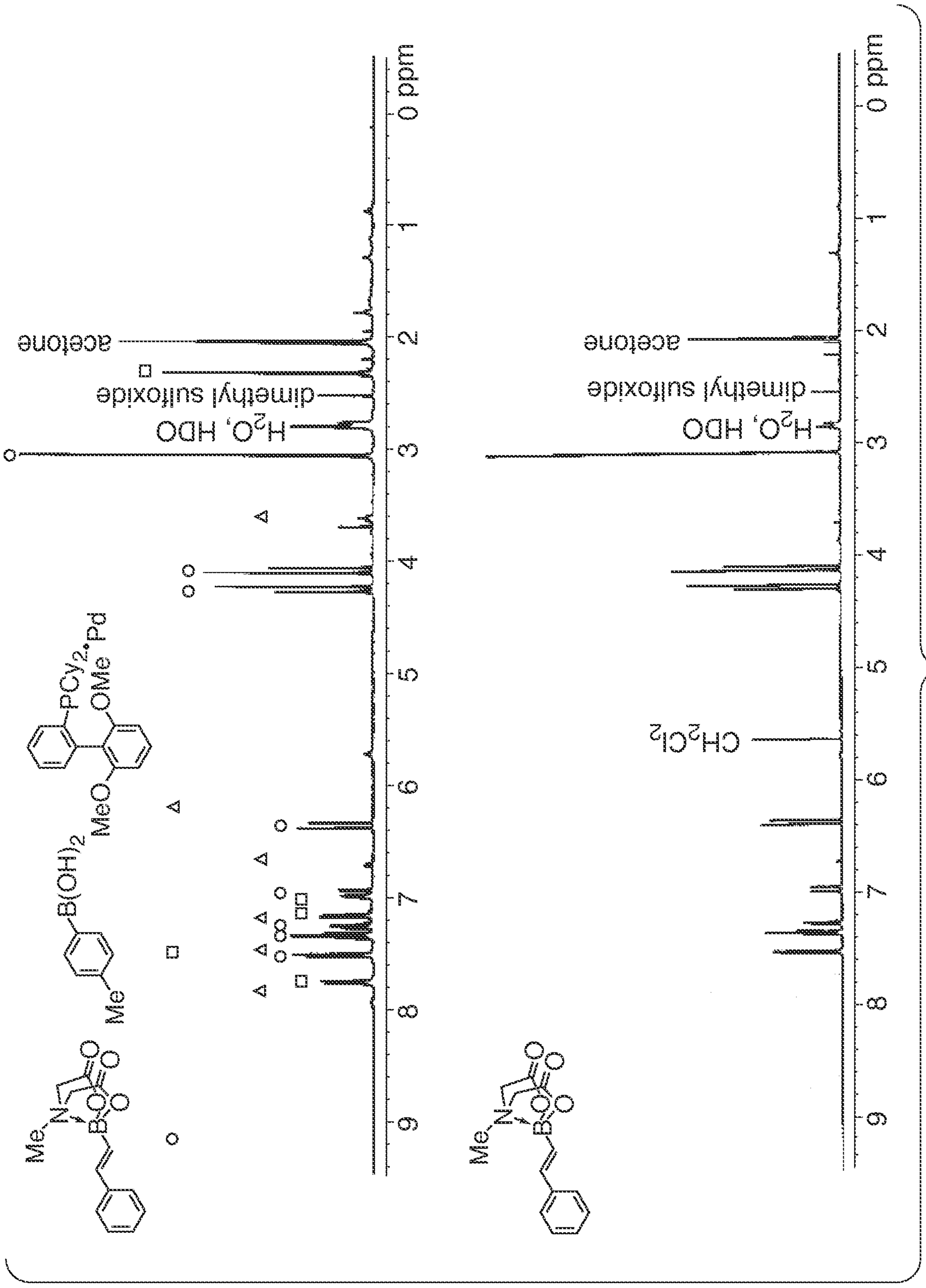

0 ppm
1
2
3
4
5
6
7
8
9
acetone
dimethyl sulfoxide
H_2O, HDO
CH_2Cl_2
Me
$B(OH)_2$
MeO
PCy_2
OMe
Pd
Me
N
B
O

Figure 26

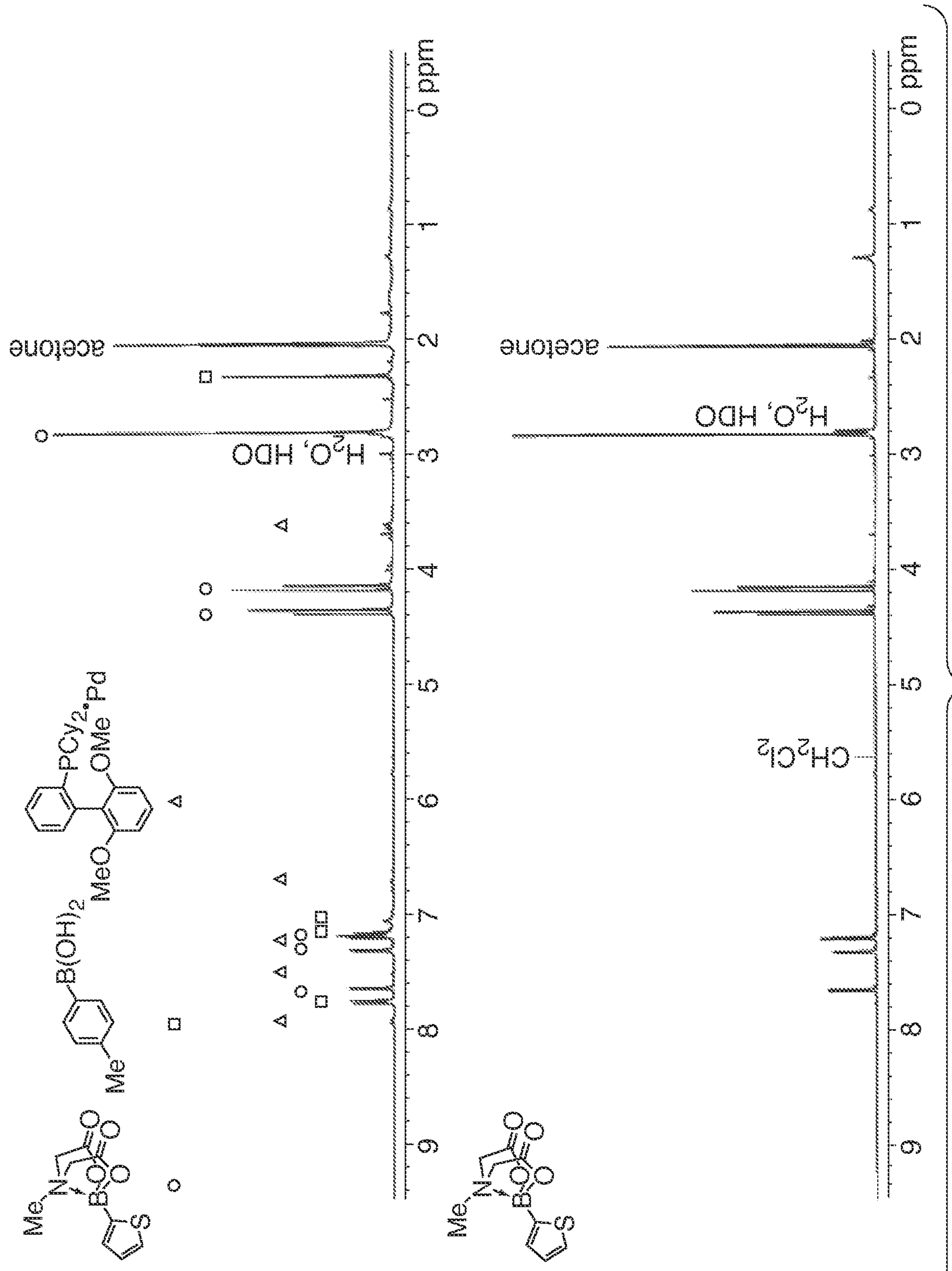

0 ppm
1
2
3
4
5
6
7
8
9
acetone
H_2O, HDO
CH_2Cl_2
Me
$B(OH)_2$
MeO
PCy_2
OMe
Pd
Me
N
B
O
S

Figure 27

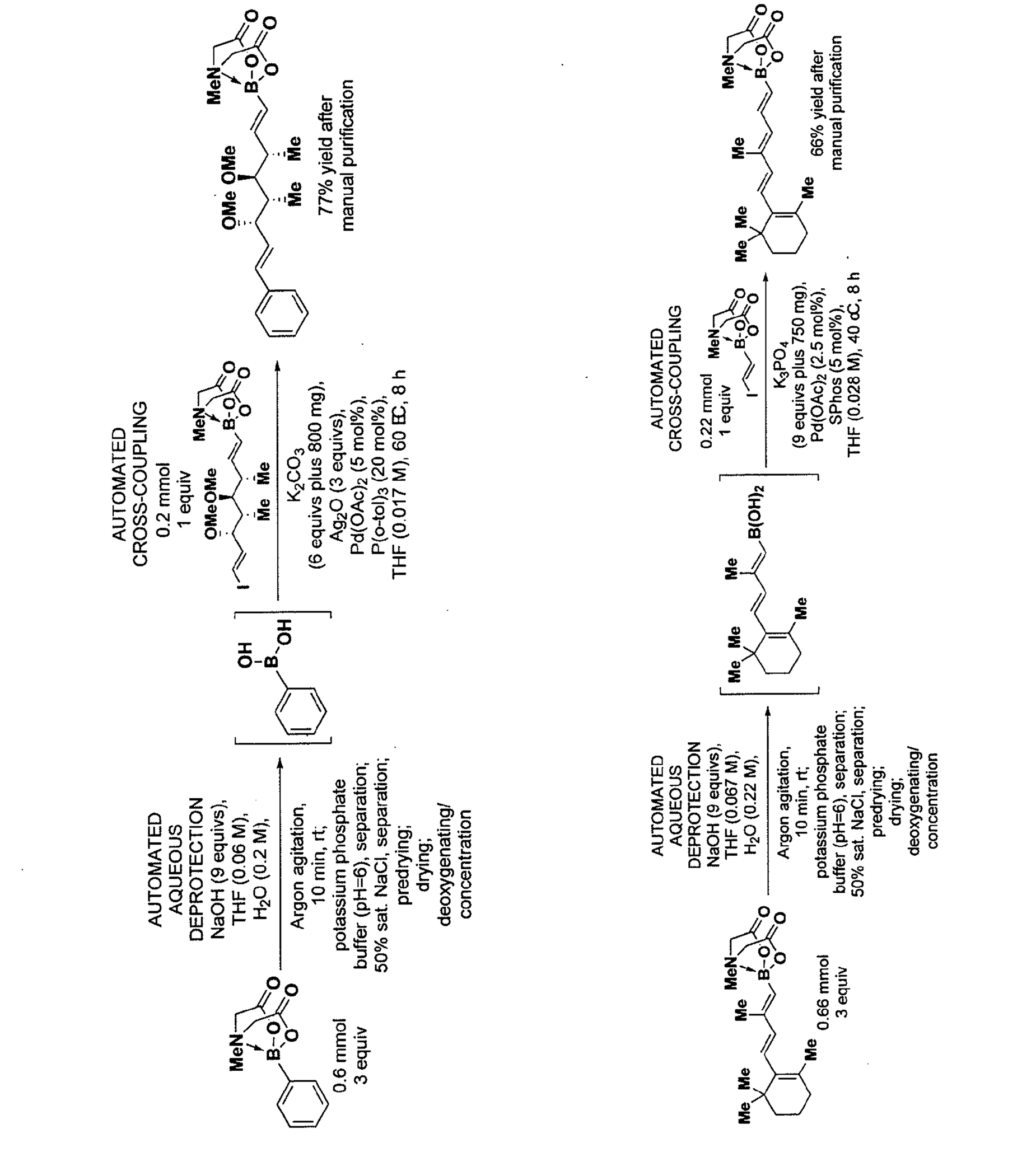
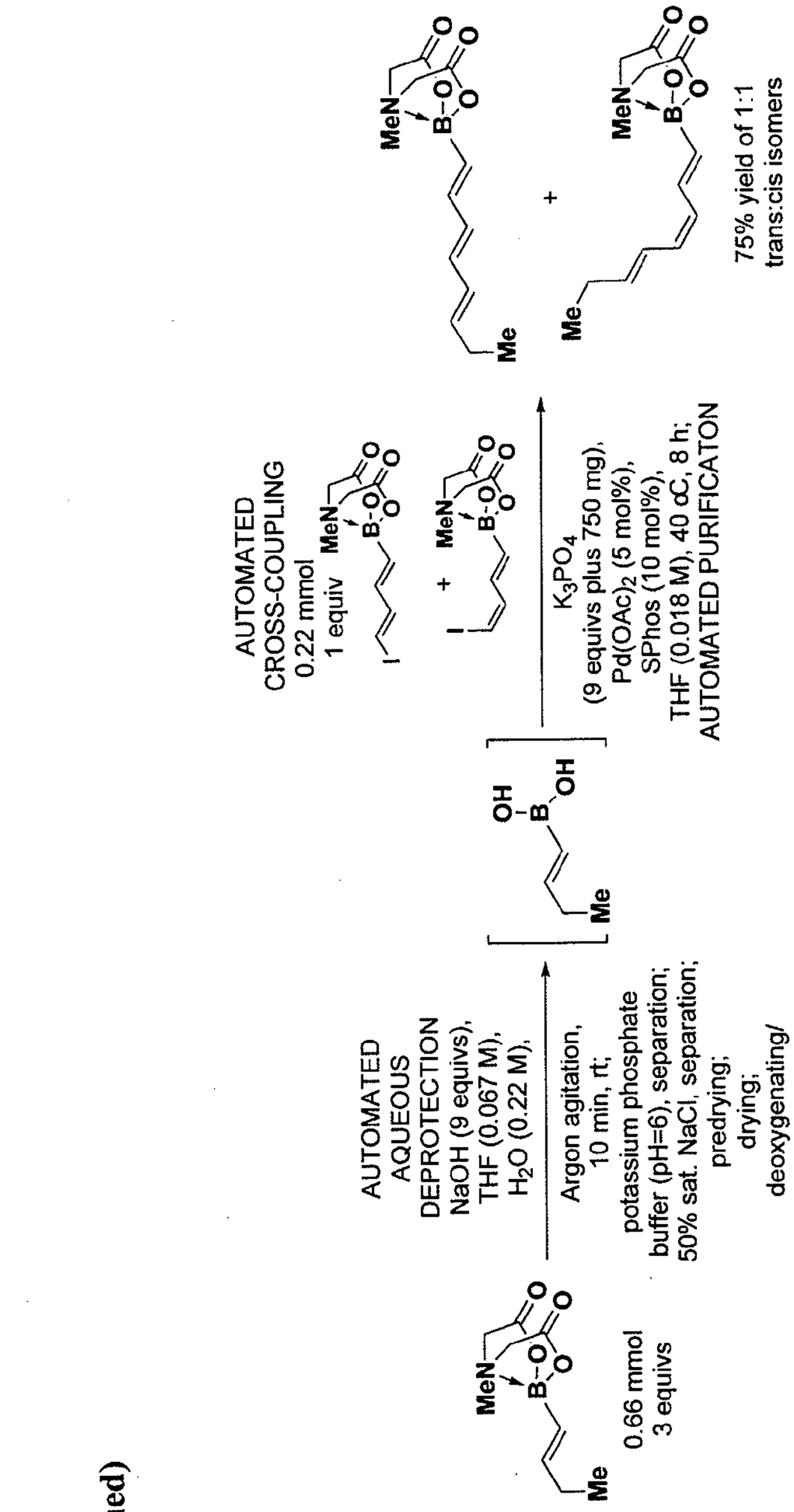

A
AUTOMATED
CROSS-COUPLING
0.2 mmol
1 equiv
OMeOMe
Me Me
I
MeN
B-O
O
O
O
K2CO3
(6 equivs plus 800 mg),
Ag2O (3 equivs),
Pd(OAc)2 (5 mol%),
P(o-tol)3 (20 mol%),
THF (0.017 M), 60 EC, 8 h
OMe OMe
Me Me
77% yield after
manual purification
AUTOMATED
AQUEOUS
DEPROTECTION
NaOH (9 equivs),
THF (0.06 M),
H2O (0.2 M),
0.6 mmol
3 equiv
Argon agitation,
10 min, rt;
potassium phosphate
buffer (pH=6), separation;
50% sat. NaCl, separation;
predrying;
drying;
deoxygenating/
concentration
OH
B
OH
B
AUTOMATED
CROSS-COUPLING
0.22 mmol
1 equiv
K3PO4
(9 equivs plus 750 mg),
Pd(OAc)2 (2.5 mol%),
SPhos (5 mol%),
THF (0.028 M), 40 oC, 8 h
Me
B(OH)2
Me
Me
Me
Me
Me
66% yield after
manual purification
AUTOMATED
AQUEOUS
DEPROTECTION
NaOH (9 equivs),
THF (0.067 M),
H2O (0.22 M),
0.66 mmol
3 equiv
Argon agitation,
10 min, rt;
potassium phosphate
buffer (pH=6), separation;
50% sat. NaCl, separation;
predrying;
drying;
deoxygenating/
concentration
B

Figure 28

Figure 28 (continued)

C



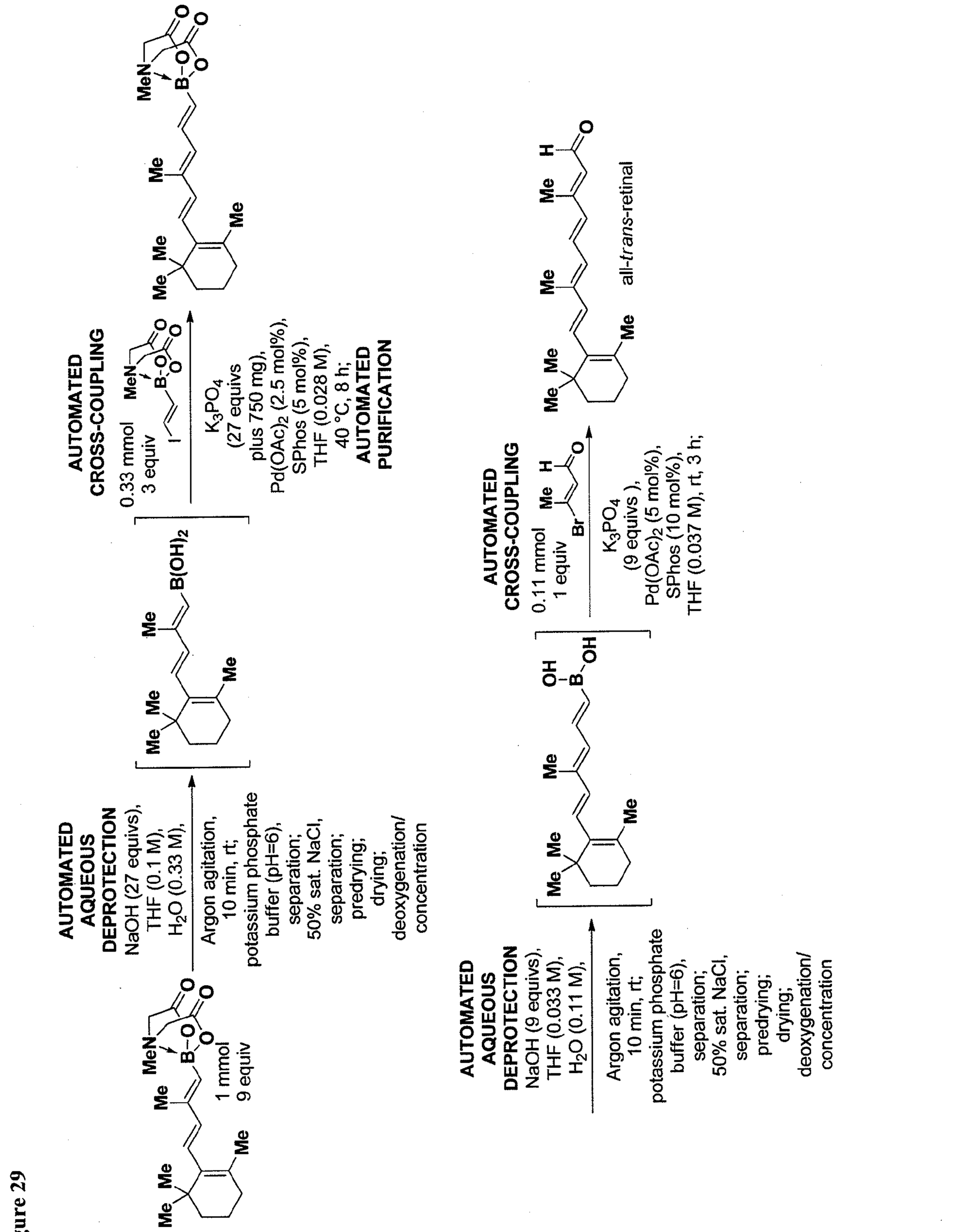

AUTOMATED
CROSS-COUPLING
0.33 mmol
3 equiv
MeN
B-O
O
O
O
I
K_3PO_4
(27 equivs
plus 750 mg),
$Pd(OAc)_2$ (2.5 mol%),
SPhos (5 mol%),
THF (0.028 M),
40 °C, 8 h;
AUTOMATED
PURIFICATION
Me
B(OH)2
Me
Me
Me
Me
Me
AUTOMATED
AQUEOUS
DEPROTECTION
NaOH (27 equivs),
THF (0.1 M),
H_2O (0.33 M),
Argon agitation,
10 min, rt;
potassium phosphate
buffer (pH=6),
separation;
50% sat. NaCl,
separation;
predrying;
drying;
deoxygenation/
concentration
1 mmol
9 equiv
AUTOMATED
CROSS-COUPLING
0.11 mmol
1 equiv
Me
H
O
Br
K_3PO_4
(9 equivs),
$Pd(OAc)_2$ (5 mol%),
SPhos (10 mol%),
THF (0.037 M), rt, 3 h;
all-*trans*-retinal
AUTOMATED
AQUEOUS
DEPROTECTION
NaOH (9 equivs),
THF (0.033 M),
H_2O (0.11 M),
Argon agitation,
10 min, rt;
potassium phosphate
buffer (pH=6),
separation;
50% sat. NaCl,
separation;
predrying;
drying;
deoxygenation/
concentration
OH
B
OH

Figure 29