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In-tube derivatization for determination of absolute configuration and enantiomeric purity of chiral compounds by NMR spectroscopy

Jinhai Gao, Srinivasan Rajan and Bing Wang*

We have developed an in-tube derivatization method using commercially available polymer-supported coupling agents to prepare derivatives of chiral compounds directly in NMR tube with high yield and purity. Because the method does not require any workup or purification, the configuration and enatiopurity can be quickly determined by NMR analysis for a small amount of chiral compounds, which is critical for today's fast-paced medicinal chemistry efforts in drug discovery. The application of the method was demonstrated for the derivatization of chiral amines, alcohols, diols, amino alcohols, thiols, and carboxylic acids using various chiral derivatizing agents and coupling agents. This article also serves as a practical guide for in-tube derivatization and selection of suitable chiral derivatizing agents and coupling agents for various types of chiral compounds. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: NMR; in-tube derivatization; polymer-supported coupling agents; absolute configuration; enantiomeric purity

Introduction

More than half of the drugs in use and most of the drugs in development nowadays are chiral molecules. Fast and efficient methods for the determination of absolute configuration and enantiomeric purity of chiral compounds have become increasingly important in supporting large number of asymmetric syntheses in modern medicinal chemistry labs. While X-ray crystallography has been a straightforward tool for the determination of absolute configuration, the requirement of a single crystal limits the routine application of this method. Chiral chromatography is currently most often used for chiral analysis. However, the application is impeded by the amount of time and resources required to develop and optimize the chiral separation method and the lack of reference compounds for many new chemical scaffolds. Optical spectroscopy, such as circular dichroism, optical rotation, and vibrational circular dichroism, has been also a useful tool for chiral analysis with some limitations.

Methods based on NMR spectroscopy for the analysis of chiral molecules are very appealing because the instrument is readily available and only a small amount of sample is needed for modern NMR instruments. NMR analysis via chiral derivatization has the additional benefit that it can provide accurate information for both the absolute configuration and enantiomeric purity of chiral compounds. In order to achieve the necessary chiral discrimination in NMR spectra of enantiomers, chiral derivatizing agents (CDAs) have been widely used to form diastereomeric derivatives for various types of chiral molecules.[1] However, the synthesis of diastereomeric CDA derivatives requires a series of time-consuming steps including reaction workup, separation, and purification. The application of the CDA-based method has not been routinely used in today's fast-paced medicinal chemistry laboratories because of the amount of material and time required to prepare the derivatives.

To avoid some of the disadvantages associated with the preparation of the CDA-substrate derivatives, methods using polymer-supported CDAs for purification free derivatization of chiral substrates have been reported.[2,3] The chiral substrates react with the polymer-supported CDAs to produce CDA derivatives in NMR solvent, and the NMR spectra can be collected without any further manipulations. The drawbacks of this method include the significant development time required to optimize the CDA-resin properties (e.g. stability and swellability in NMR solvent, reactivity, and regioselectivity with the chiral sustrate), and limited choices of CDAs that can be applied. We have successfully implemented the 'mix and shake' method with methoxyphenylacetic acid (MPA) as the CDA,[3,4] but it did not always give satisfactory results when we tried to expand the method to some other CDAs [e.g. methoxytrifluoromethylphenylacetic acid (MTPA)]. Because of the lack of commercial availabilty of polymer-supported CDAs, this method does not offer the speed and flexibility needed in a drug discovery enviroment.

Herein we report that by switching the polymer-supported reagent to the coupling reagent, rather than the CDA, a more versatile and efficient method to carry out the derivatization procedure directly in NMR tube is achieved. This in-tube derivatization method does not require the preparation of CDA-resins beforehand; as a result, we can screen many types of CDAs with a range of commercially available polymer-supported coupling reagents in order to obtain optimal chiral discrimination and accurate enatiopurity measurements for various types of chiral compounds.

* *Correspondence to: Bing Wang, Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Inc., Cambridge, MA 02139, USA.*
E-mail: bing.wang@novartis.com

Global Discovery Chemistry, Novartis Institutes for Biomedical Research, Inc., Cambridge, MA, 02139, USA

Results and Discussion

General methodology and procedure of in-tube derivatization method

Most commonly used CDAs have carboxylic acid groups, which will be linked to the functional groups from chiral substrates. The synthesis is usually initiated by an coupling reagent forming a reactive intermediate with the CDA. The chiral substrate acts as a nucleophile attacking the reactive intermediate to form the desired CDA derivative. The development of in-tube derivatization method is based on the following ideas: (i) the polymer-supported coupling agent is used for the coupling of CDA and chiral substrates directly in NMR tube in deuterated solvent; (ii) the polymer-supported coupling agent and any by-products remain out of the solution (afloat on top of the NMR solvent such as $CDCl_3$) and the NMR detection coil; and (ii) clean NMR spectra of the desired derivatives can be obtained directly in the same NMR tube without any other manipulations.

Because the NMR spectra are collected directly in the NMR tube without any separation or purification, complete conversion of the original chiral compounds is important in order to avoid signal overlaps between any unreacted compounds and the desired products. Peak overlapping makes it difficult to accurately measure the chemical shifts and integrals, which are critical for reliable determination of absolute configuration and enantiomeric purity. The complete conversion is also critical for accurate measurement of the enantiomeric purity because of the effect of kinetic resolution. If the conversion is incomplete, the ratio of the diastereomeric CDA derivatives will be affected by the relative reaction rate of enantiomers with the CDA, potentially resulting in the kinetic resolution of the products, leading to inaccurate chiral analysis. The kinetic resolution will have no influence on the final ratio of CDA derivatives provided that all chiral substrates are completely converted. The real-time monitoring of the in-tube derivatization procedure directly in NMR tube is superior to the traditional synthesis approach, because it ensures the complete conversion of the chiral substrate. The purification procedure involved in the traditional synthesis also introduces errors in the measurements because of the various degree of sample loss.

In order to achieve complete transformation of the original chiral samples, excess amount of CDA and coupling agent are used for the in-tube derivatization procedure. The extra CDA reacts with the excess amount of coupling agent on the resin and remain out of the NMR detection coil. The typical procedure for in-tube derivatization can be described as follows: (i) appropriate amount of polymer-supported coupling agents (2 equiv relative to chiral substrate) is weighed in NMR tube and NMR solvent (e.g. $CDCl_3$) was added to soak the resin. (ii) The CDA (1.5 equiv) is added and mixed with the resin. (iii) The chiral substrate (1.0 equiv) is then added, and the mixture is shaken either by hand or on a shaker if long reaction time is required. (iv) NMR spectra are collected directly from the same NMR tube to monitor the reaction until it is completed. With the advantages of complete conversion and no sample loss during the in-tube derivatization procedure, the amount of chiral compound required is minimal as long as good quality ^{1}H NMR spectra can be obtained. On a modern high field NMR instrument with cryo-probes, this can be achieved for as low as micrograms of samples.

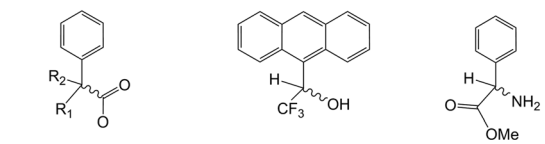
During the optimization of the in-tube derivatization procedure, we found that the presence of water in the reaction mixture is the main reason for incomplete conversion and long reaction time. This can lead large errors in enantiomeric purity measurement because of kinetic resolution. The water impurity could come from all the components that are added in the NMR tube, and it is impractical to keep all the materials completely dry before the derivatization procedure. We found that adding some molecule sieve beads directly into the NMR tube is very effective to remove small amount of water from the reaction mixture. The molecular sieves will either stay at the bottom of NMR tube or float with the resin on $CDCl_3$ without interfering with the NMR data collection.

Racemization of either CDA or the chiral substrate is another important concern for the in-tube derivatization procedure. Fortunately, many more efficient coupling agents have been developed and become commercially available in polymer-supported format. The flexibility in selection of coupling agents and CDAs for the in-tube derivatization procedure allows the optimization of reaction conditions so that racemization or other side reactions can be avoided or minimized. Examples will be discussed in the application sections to demonstrate how the racemization products were easily identified and quantified by NMR, and the reaction conditions were optimized to minimize the racemization.

In-tube derivatization of chiral amines

Methoxytrifluoromethylphenylacetic acid (MTPA),[5,6] MPA,[7] and Boc-phenylglycine (BPG)[8] (Scheme 1) are popular CDAs for chiral NMR analysis of amines and alcohols. Polymer-supported dicyclohexylcarbodiimide (PS-DCC) has been widely used as the coupling agent for the preparation of CDA derivatives.[9] The in-tube derivatization procedure was successfully carried out for the transformation of amine **1** (Scheme 2) to (*R*)- and (*S*)-MPA amides using PS-DCC. Monitoring the ^{1}H NMR directly in the tube (Fig. 1 (a)), we observed full conversion in about 15 min. The absolute configuration and enantiomeric purity (98% measured vs 99% reported) of **1** can be determined from the ^{1}H NMR spectra of (*R*)- and (*S*)-MPA amides (Fig. 1(b)). Racemization and kinetic resolution are rarely observed for derivatization of primary amines as the complete conversion can be easily achieved in a very short period of time. Excellent results were also obtained for the in-tube derivatization of chiral amine **1** and **2** using MTPA and BPG as the CDA.

MTPA has been used as CDA for the configurational assignments of cyclic secondary amines.[10,11] Because of the steric hindrance between secondary amine and MTPA, extended reaction times are required to reach full conversion. The transformation of secondary amine **3** with (*R*)- and (*S*)-MTPA was completed within 2 h using PS-DCC without any noticeable racemization. It is worth noting that MTPA derivatives of secondary amines with very bulky subsitutions are very difficult to make from our experience using the mix and shake method. As an alternative to MTPA, we have used MPA successfully to prepare the amide derivatives of a sterically hindered

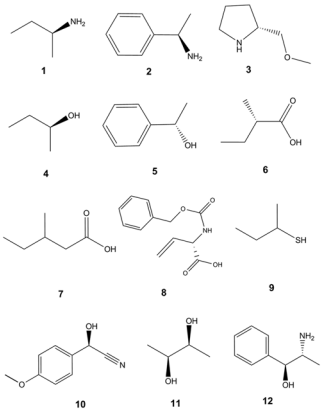


MPA: R1=OMe, R2=H
MTPA: R1=OMe, R2=CF_3
BPG: R1=NBoc,R2=H
OAM: R1=OAc, R2=H

TFAE

PGME

Scheme 1. Structures of CDAs.



Scheme 2. Structures of compounds **1–12**.

cyclic sencodary amine and established the conformational model and correlation between the large shielding effects and the configuration.[4]

In-tube derivatization of chiral alcohols

Derivatization of alcohols is more difficult than amines, so 4-(dimethylamino)pyridine (DMAP) is commonly used as a catalyst in the coupling reaction. Although the NMR signals of free DMAP are present in the NMR spectrum, these signals can be easily identified and usually do not interfere with the NMR data analysis. PS-DCC resin with DMAP (0.2 equiv) was successfully employed for the in-tube derivatization of **4** and **5** with (*R*)- and (*S*)-MPA. The complete transformations of **4** and **5** to the corresponding MPA esters were achieved within 1–3 h without racemization, allowing the accurate determination of chiral purity of the original chiral alcohols. Figure 2(a) showed the transformation of **4** with (*R*)-MPA as monitored by NMR. The enantiomeric purity determined from the ratio of diastereomeric (*R*)- and (*S*)-MPA esters of **4** was 90% (Fig. 2(b)), which is in good agreement with the purity of 91% reported from the supplier . The enantiomeric purity of **5** was 98% from NMR analysis *versus* the reported purity of 99.5% .

O-Acetylmandelic acid (OAM) has been reported to be a more reliable CDA for the configurational determination of some chiral alcohols, especially sterically crowded alcohols.[12] In-tube derivatization of **4** and **5** with OAM was conducted using PS-DCC/DMAP. However, enantiomeric purities measured from the OAM esters prepared by PS-DCC were much lower (80% for **4**; 86% for **5**) than those measured from MPA esters, suggesting that racemization of OAM (supplied chiral purity 98%) occurred under the reaction condition. In search for an alternative derivatization method, we tested the more activated polymer-supported *N*-alkyl-2-chloro pyridinium triflate (PL-Mukaiyama).[13] For the derivatization of alcohols using PL-Mukaiyama, an access amount of diisopropylethylamine (DIEA, 2 equiv) and a catalytic amoun of DMAP (0.2 equiv) were used to facilitate the coupling reaction. We tested different reaction conditions using either PS-DMAP with free DIEA or PL-DIEA with free DMAP and found that the later combination gave better yield and shorter reaction time with additional advantage that only small NMR signals from free DMAP are present in the final ^{1}H NMR spectra. The enantiomeric purities determined from the OAM esters prepared by PL-Mukaiyama were consistent with those reported for **4** (88% vs 91%) and **5** (96% vs 99.5%). It is recommended that PS-DCC should be avoided when OAM is the preferred CDA for in-tube derivatization.

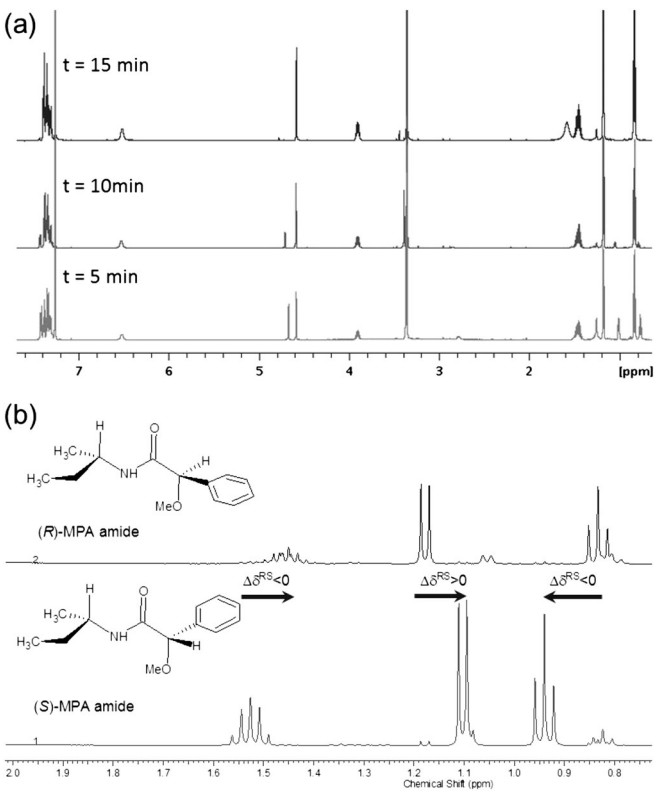


Figure 1. (a) Transformation of **1** with (*R*)-MPA using PS-DCC monitored by NMR. (b) ^{1}H NMR spectra of (*R*)- and (*S*)-MPA amides of **1**.

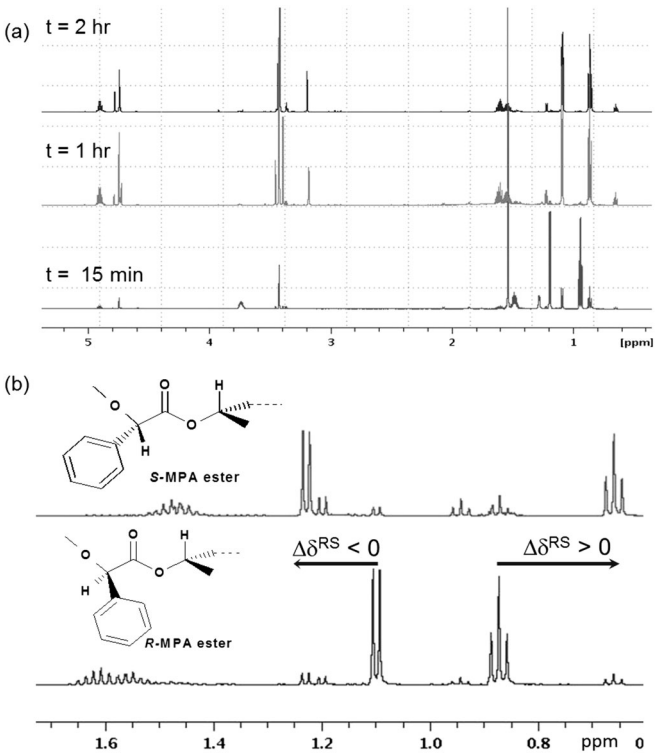


Figure 2. (a) Transformation of **4** with (*R*)-MPA using PS-DCC monitored by NMR. (b) ^{1}H NMR spectra of (*R*)- and (*S*)-MPA esters of **4**.

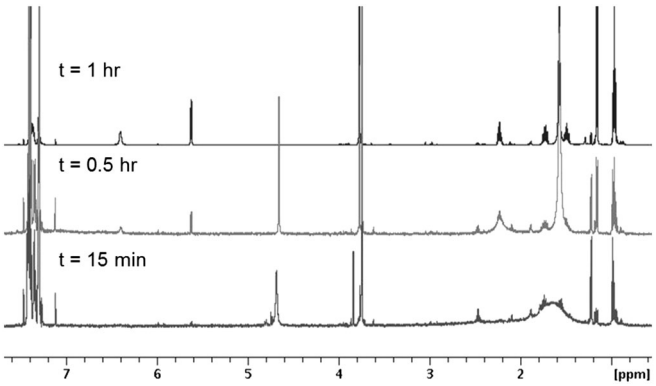
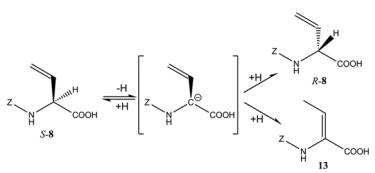


Figure 3. Transformation of **6** with (*R*)-MPA using PS-DCC monitored by NMR.

In-tube derivatization of chiral carboxylic acids

Phenylglycine methyl ester (PGME)[14,15] and 1-(9-anthryl)-2,2,2-trifluoroethanol (TFAE)[16] have been used as the CDAs for NMR analysis of chiral carboxylic acids. Different from the in-tube derivatization procedure discussed in the preceding texts, the chiral carboxylic acid now first reacts with the polymer-supported coupling agent to form the active intermediate, and the CDAs (PGME is an amine and TFAE is an alcohol) act as nucleophiles during the coupling reaction. The modified procedure was used for the preparation of PGME amide of **6** and TFAE ester of **7** using PS-DCC. Figure 3 shows the transformations of **6** to the corresponding (*R*)-PGME amide using PS-DCC. We used the same equivalence of CDA in this example to evaluate the extent of conversion while minimizing the NMR signals from unreacted PGME. The conversion of chiral carboxylic acids **6** was estimated to be >90%, which is sufficient for configuration determination but may cause error for enatiopurity measurement because of potential kinetic resolution.

The real-time NMR monitoring during the in-tube derivatization procedure not only provide quantitative measurement of reaction components but also provide detailed structural information and understanding of the reaction mechanism to guide the optimization of reaction conditions. This was demonstrated by the in-tube derivatiozation of chiral carboxylic acid **8**, which is prone to epimerization and isomerization. Not surprisingly, when PS-DCC was first used for the in-tube derivatiozation of **8** with (*S*)-PGME, a mixture of three components with ratio of 4 : 3 : 3 were observed in ^{1}H NMR spectrum (Fig. 4(a)). Detailed analysis of the NMR data revealed that the mixture is the (*S*)-PGME esters of the following products: the original (*S*)-**8**, racemization product (*R*)-**8**, and the olefin isomer **13** (Scheme 3). The racemization was believed to occur during the activation step of the carboxylic acid with DCC. 2-Isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ) has the advantage over conventional carbodiimide-based coupling reagents that no pre-activation step is required, and therefore, the acid can be added at the last step in order to minimize the chance of racemization. When PL-IIDQ[17] was used as the coupling agent for the in-tube



Scheme 3. Racemization and olefin isomerization of **8** during amide coupling with (*S*)-PGME using PS-DCC.

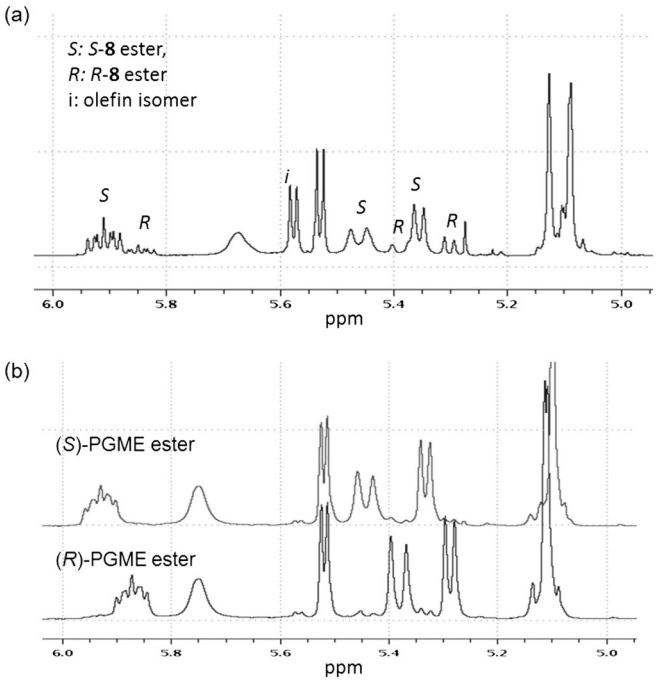


Figure 4. (a) ^{1}H NMR spectrum of in-tube derivatization products of **8** and (*S*)-PGME using PS-DCC. (b) ^{1}H NMR spectra of (*R*)- and (*S*)-PGME amides of **8**.

derivatization of **8**, a single product was obtained for each (*R*)- and (*S*)-PGME amides of **8** (Fig. 4(b)), allowing unambiguous determination of the configuration and chiral purity of **8**. PL-IIDQ proved to be a very efficient coupling agent with minimal racemization of the chiral carboxylic acid. The drawback is the release of isobutanol, which will show NMR signals when it is used for in-tube derivatization.

In-tube derivatization of other chiral functional groups

The CDA method has been also applied to other chiral functional groups such as thiol, diol, and amino alcohol. The in-tube derivatization of thiol (**9**), cyanohydrin (**10**), and diol (**11**) with MPA using PS-DCC was carried out successfully with excellent yield. Interestingly, we found that the derivatization of amino and hydroxyl groups in **12** with MPA can be achieved in two separate steps. First, the amine group was transformed to MPA amide using PS-DCC by following the derivatization procedure for primary amines. The amidation of amine group was selective, and no transformation of hydroxyl group was detected in ^{1}H NMR spectrum. The configuration of the chiral amine can be determined by comparison of the NMR spectra of (*R*)- and (*S*)-MPA amides. Second, the same NMR sample with either (*R*)- or (*S*)-MPA amide was further transformed to MPA esters of the hydroxyl group when additional MPA and PS-DCC/DMAP were added to the NMR tube. The configuration of the hydroxyl group can be determined by comparison of the NMR spectra of (*R*)- and (*S*)-MPA esters. This step-by-step derivatization method avoid the complication of analyzing the bis-MPA derivatives for which the chemical shift changes are complicated by the 'combined shielding effects' of MPA amide and ester.[18]

Conclusion

A fast and efficient in-tube derivatization method has been developed for the determination of absolute configurations and enantiomeric purity of chiral compounds. Common problems such as

racemization and incomplete conversion during the coupling reaction were overcome by optimization of the reaction conditions and selection of optimal coupling agents. The application and optimization of the in-tube derivatization method were successfully demomstrated for various types of chiral functional groups and coupling agents in $CDCl_3$. Other NMR solvents such as dicholoromethene-*d2* and aceton nitrile-*d4* have been commonly used in resin-based synthesis and are also good choices for the in-tube derivatization. The procedure is easy to implement and can be conducted quickly in any chemistry or analytical NMR laboratories. With the availability of today's highly sensitive NMR instruments, the method can be used to determine the absolute configuration and enantiomeric purity of chiral compounds at microscale level.

Experimental

Materials

All chiral amines, alcohols, carboxylic acids, and chiral derivative agents were ordered from Sigma-Aldrich or Fluka (Milwaukee, WI). PGME was supplied as HCl salt, which was removed by using PL-HCO3 MP cartridge. Polymer-supported coupling agents PS-DCC and PS-DMAP were purchased from Biotage (Biotage AB, Sweden). PL-Mukaiyama, PL-IIDQ, PL-DIEA were purchased from Polymer Laboratories (Amherst, MA). $CDCl_3$ was purchased from Cambridge Isotope Laboratories (Andover, MA).

NMR spectroscopy

NMR spectra were recorded on a Bruker AVANCE NMR spectrometer operating at a frequency of 600.13 MHz equipped with a 5 mm TCI probehead with *z*-gradient. All spectra were recorded at 300 K, and the chemical shifts for ^{1}H NMR spectra were referenced to internal tetramethylsilane at 0 ppm.

In-tube derivatization using PS-DCC

Seventeen milligrams of PS-DCC (0.02 mmol, 1.2 mmol/g loading, 2 equiv) was weighted in a 5 mm NMR tube, and 0.6 ml of $CDCl_3$ was added to soak the resin for 10 min. The cleanness of the resins can be checked by ^{1}H NMR spectrum, and the resins can be washed with additional $CDCl_3$ to remove any impurities from the resin if necessary. The carboxylic acid CDA (0.015 mmol, 1.5 equiv) in $CDCl_3$ solution was added and mixed with the resin for 10 min. Chiral amine or alcohol substrate (0.01 mmol, 1 equiv) in $CDCl_3$ solution was added, and the NMR tube is shaken either by hand or on a shaker if long reaction time is necessary. NMR spectra were collected directly from the same NMR tube to monitor the reaction until it is completed. For the derivatization of alcohol, DMAP (0.002 mmol, 0.2 equiv) was added to catalyze the reaction.

In-tube derivatization using PL-Mukaiyama

Nineteen milligrams of PL-Mukaiyama (0.02 mmol, 1.1 mmol/g loading, 2 equiv) with PL-DIEA (0.02 mmol, 6 mg, 3.5 mmol/g loading, 2 equiv) was added in a 5 mm NMR tube, and 0.6 ml of $CDCl_3$ was added to soak the resin. DMAP (0.002 mmol, 0.2 equiv) was used to catalyze the derivatization of alcohol (0.01 mmol, 1 equiv) in $CDCl_3$, following the same procedure as described in the preceding texts.

In-tube derivatization using PL-IIDQ

Twelve milligrams of PL-IIDQ (0.02 mmol, 1.7 mmol/g loading, 2 equiv) was weighted in a 5 mm NMR tube, and 0.6 ml of $CDCl_3$ was added to soak the resin for 10 min. PGME (0.01 mmol, 1 equiv) was added followed by carboxylic acid **8** (0.01 mmol, 1 equiv). The reaction was monitored by ^{1}H NMR spectroscopy until it's completed.

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