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WHITE PAPER

# Ten Things You Should Know Before Contracting a Custom Synthesis Project

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## INTRODUCTION

Companies often need to arrange for the synthesis of test substances to support research and development efforts.

There are a number of important decisions to consider before proceeding with a contract for synthesis. An adaptation of the "Five W's" for gathering information and problem solving—Who, What, When, Where, and Why—provides a good framework for decision-making:

**Who.** Who do you need to talk to about your synthesis project? That depends very much on your specific needs and scale, which can range from small quantities of a reference standard to support a bioanalytical project to much larger amounts of API to support a clinical trial. The quality agreements, production systems, and contracting arrangements are quite different across that range.

For large scale projects, you will want to consult with a large-scale Contract Manufacturing Organization (CMO).

This white paper provides advice and guidance to those with the responsibility of procuring the milligram to multi-gram amounts of reference standards, internal standards, impurities, or radiolabeled materials produced by custom synthesis vendors.

**What.** What kind of material do you need? $^{14}C$ or $^{3}H$ for a metabolism study? 12C for a reference standard? $^{13}C$, $^{2}H$, or $^{15}N$ for an internal standard?

**When.** It's important to allow sufficient lead time for the procurement of starting material, the actual synthesis, and any other deliverables (such as a Certificate of Analysis). As the complexity of a synthetic scheme increases, whether in chemistry or steps, so does the time required. Timing also will depend on the regulatory approach: for example, CGMP synthesis will take significantly longer than a non-regulatory project.

**Where.** For radioactive materials, it's especially important to decide where on the molecule the label should be incorporated. This can sometimes require the design of a new synthetic pathway. Depending on the molecule, radiolabeling in multiple positions—or the strategic use of multiple isotopes—may assist in defining the metabolic pathway.

**Why.** It's last in the Five W's, but this should always be the *first* question you ask before proceeding with a custom synthesis. Good study design should always come first to avoid complications later. Is the test substance to be used in humans or in preclinical species? Is the material needed for tracking degradation in plants, animals, and/or environmental matrices? Answers to these questions have important implications for regulatory requirements and material needs.

With the basic questions answered above, here are ten other important topics to consider before proceeding with a custom synthesis program.

## 1. COMMUNICATION

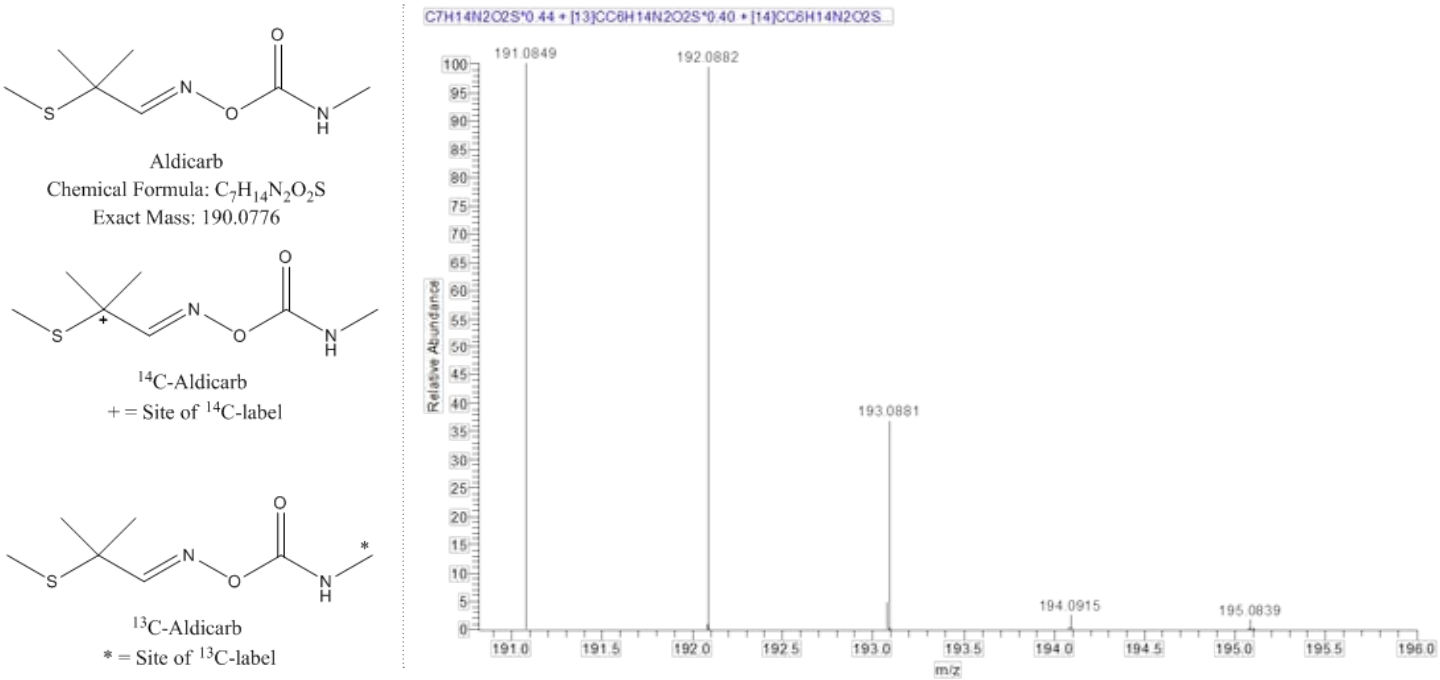
Good communication is key to any successful sponsor-contractor relationship. For custom synthesis, it's especially important as the test substance supply is a critical material for the studies it supports. On-time delivery of a high quality substance is a *minimum* expectation; on top of that, a good CRO will want to be a partner in your ongoing program. It's important to take time in the proposal process to discuss your study design, material and timing needs, regulatory requirements, institutional knowledge on the chemistry or synthesis pathways, future supply needs, and more. Once a synthesis campaign is underway, the communication should certainly continue with frequent updates on progress.

## 2. REGULATORY REQUIREMENTS

The consideration of regulatory requirements in your custom synthesis program is very important and should be made as early as possible in the process. The choice can affect timing and cost, but the most important factor is how it matches with your own study design and regulatory needs. There are three main options: non-regulatory, GLP and GMP:

**Non-Regulatory.** If the material is to be used for laboratory research purposes only, then a non-regulatory synthesis program and Certificate of Analysis (CoA) should suffice.

**GLP (Good Laboratory Practices).** It is important to note that




**When combined in an approximate 1:1:0.3 ratio, a blend of $^{13}$C and $^{14}$C isotopes in different parts of the molecule produces this mass spectrum.**

GLP guidelines do not apply to the production of a test substance, so the synthesis itself will not be conducted under GLP (i.e., there is no such thing as a "GLP lot"). However, they do apply to the characterization, handling, and storage of test and control articles. To that end, the CoA will be done under the auspices of a protocol, with a study director and Quality Assurance Unit (QAU) review of data and procedures. The lack of a GLP CoA, where one is expected, may result in a statement of non-compliance in a GLP study report.

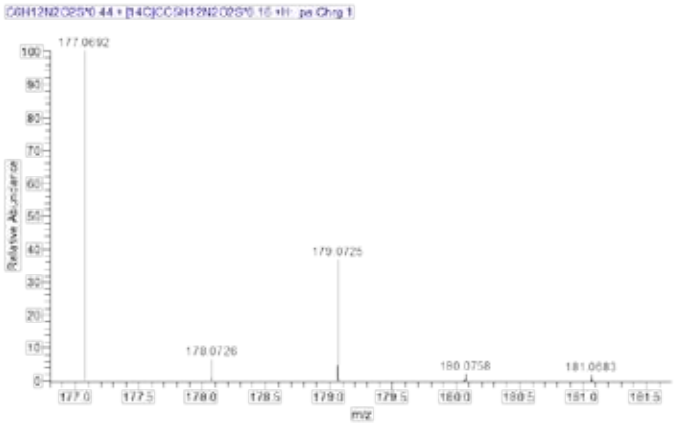
**CGMP (Current Good Manufacturing Practices).** Unlike GLP, CGMP guidelines are concerned with both production and quality control. Effective, timely, and quality production of radiolabeled Clinical Trial Materials (CTMs) requires radiochemistry expertise and rigorous CGMP-compliant facilities, processes and systems. Once synthesized, radiolabeled drugs often exhibit different stability and impurity profiles; to ensure the validity of studies, these variances must be understood. These and other issues present special challenges related to CGMP compliance (and patient safety) during the clinical trial.

Be certain to inquire about the laboratory's inspection record with regulatory authorities; you may also want to schedule your own technical and/or quality assurance audits.

## 3. STRATEGIC USE OF ISOTOPES

There a variety of isotopes to choose from to meet your study design and needs. The use of the material in the study will determine the types of isoptope(s) to be used: i.e., radiolabeled material for metabolism studies, stable labels for internal standards, etc.

Generally, $^{14}$C is the label of choice for most molecules, as it can be introduced into a metabolically stable position (see "Position of Radiolabel"). With 14C, detection is straightforward and specific activities offer good stability. In contrast, $^{3}$H-labeled substances can generally be prepared more quickly, but the $^{3}$H-label is more prone to instability (from higher specific activities) and formation




**However, if the N-methyl group of this blend was lost, the spectrum would change dramatically due to the loss of the increased amount of $^{13}$C label; however, the amount of $^{14}$C still allows the structure to be clearly assigned.**

of $^{3}H_2O$ during metabolism. Additionally, facilities incorporating reactions that employ tritium gas for processes such as double-bond reductions and aromatic tritio-dehalogenation reactions are often purpose-built for handling multi-curie amounts of tritium precursors and waste products.

Stable isotope-labeled compounds (using $^{2}$H, $^{13}$C, $^{15}$N, etc.) are increasingly used as internal standards in bioanalytical methods and are frequently blended with radioisotopes for metabolism studies. This latter point is extremely useful for mass spectroscopists to ensure that the correct mass is being assigned for minor metabolites in complex biological matrices.

In your study design, you may want to consider the use of multiple isotopes: a blend of radio- and stable-labels. Indeed, the increased use of high resolution mass spectrometry (HRMS) in metabolism studies, for example, allows for the strategic use of the isotope pattern filters (IPF) in the HRMS software for data mining and structural elucidation. Consider the utility of a blend of $^{13}$C and $^{14}$C isotopes in different parts of the molecule in the

example shown above.

When they are blended in an approximate 1:1:0.3 ratio, you get the mass spectrum show at the top of the page.

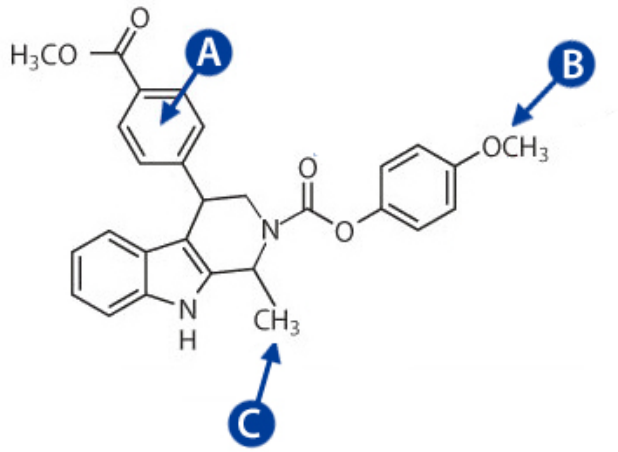
Any metabolite where the S-methyl moiety is lost will retain the same spectral pattern as shown above; for example, if the sulfur was oxidized, the pattern would still look similar. However, if the N-methyl group was lost (and any other metabolites associated with this loss), the spectrum (below) would change dramatically due to the loss of the increased amount of $^{13}C$ label; however, the amount of $^{14}C$ still allows the structure to be clearly assigned.

A good laboratory will offer advice on the benefits of the strategic use of multiple isotopes.

## 4. POSITION OF RADIOLABEL

Determination of the optimal position of the radiolabel in the test substance is a critical decision point before proceeding with the synthesis. Ideally, the radiolabel should be incorporated in the molecule at a metabolically stable site (or multiple sites, for complicated chemistry), so that it will be conserved in the degradation products observed in plasma, tissue, excreta or environmental matrices.

It is also important to ensure that radiolabels are not placed in positions that are readily converted to natural products, since these labels can lead to high amounts of bound or non-




**Ideally, the radiolabel should be incorporated in the molecule at a metabolically stable site (or multiple sites, for complicated chemistry), so that it will be conserved in the degradation products observed in plasma, tissue, excreta, or environmental matrices.**

extractable residues that are difficult to identify.

It's important to note, however, that incorporating the radioactive isotope into the test substance sometimes involves developing a new synthetic pathway. You will want to ensure that your contract synthesis laboratory has the expertise to provide advice on preferred radiolabel position(s) and suggest the merits of possible synthetic pathways.

## 5. TIMING

It's important to communicate your timing expectations for the custom synthesis as early as possible in the contracting process; likewise, it's important to plan ahead and allow sufficient time for the synthesis and testing to be completed to meet those expectations. There are (at least) four main considerations when it comes to timing:

**Laboratory Capacity.** Lead times at custom synthesis laboratories may vary based on current commitments.

**Starting Materials.** Allow at least one to two weeks for procurement of starting materials; novel starting materials may take longer to secure.

**Synthesis.** Timing is generally driven by the number of steps in the synthesis and/or challenging chemistry. You can expect one to two-step syntheses to take one to three weeks; synthesis schemes with eight to 10 steps may take 10 to 12 weeks or longer.

**Testing.** When the synthesis is completed, the material should be characterized for purity and identity to ensure it meets your specifications. A non-regulatory certification may take as little as one to two weeks.

Regulatory certifications (GLP or CGMP) generally take longer to allow time for testing and review by the Quality Assurance Unit. For CGMP synthesis, especially, developing the batch record and the regulatory requirements for second party verification add significant time and effort.

A good laboratory will provide you with a proposed schedule.

## 6. SCALE-ABILITY

Laboratory-scale syntheses are not always easily scalable to process scale (multi kilogram), and vice versa, in terms of yield, safety, time or cost. For instance, a laboratory-scale synthesis may call for the use of butyllithium at -78˚C for a metalation reaction which is transferred via cannula to another reactor held at -45˚C. These reaction conditions may be required to minimize a side reaction or provide desired stereo-selectivity. This procedure is feasible for gram scale quantities, but becomes challenging at the multi kilogram scale and is completely impractical at a commercial scale.

Purification is another consideration for scale-ability. As an example, at the milligram scale, material can be purified by reverse phase chromatography. However, this is impractical for kilograms of product.

Lab scale routes that efficiently lead to multiple analogs may not be a good fit for a process that needs to produce a single chemical entity with the same efficiency. A good custom synthesis laboratory will be able to provide advice on the suitability of the scheme that you provide.

## 7. REPURIFICATION AND CHARACTERIZATION

Perhaps you already have material on hand but are unsure of its purity. Many laboratories will assess aged materials for purity and identity and can repurify the material to needed specifications. Laboratories that offer regulatory services can also certify the material to GLP regulations. A good laboratory will also offer to conduct a specific stability test (perhaps short-term at an elevated temperature) to accurately assign an expiration date. It's important to note that it is not possible to retroactively certify material to CGMP standards—per the FDA, CGMP quality has to be built in from the beginning; it cannot just be applied at the end. As noted above, decisions on regulatory considerations should be made early in the process.

In addition to characterization of purity and identification, another important consideration is stability. There is often

insufficient information on the stability of compounds, especially new chemical entities. The stability of non-radiolabeled standards can be evaluated in long-term or accelerated conditions. Radiolabeled compounds—especially those of high specific activity—may be subject to unique stability issues, often have shorter shelf-lives, and should be evaluated for stability on their own.

## 8. METABOLITE AND IMPURITY REFERENCE STANDARDS

Owing to the regulatory landscape associated with metabolite profiling, the quantification of metabolites—in addition to their characterization and identification—has become an important part of the drug and agricultural chemical development process.

There are several drivers for synthesis of reference standards, including, but not limited to, avoiding the unsatisfying assumptions that are necessarily associated with semi-quantitative approaches to metabolite profiling; the need to screen metabolites for drug target activity in established bioassays; and the need for sufficient metabolite exposure in toxicity and other testing (drug transport, drug-drug interactions, etc.).

Chemical synthesis is often suitable for phase I metabolites such as dealkylations, oxidations, and carbonyl reductions, etc. However, novel metabolites, especially phase II metabolites, may not be synthetically accessible at the bench owing to difficult routes, stereo-selectivity, and or/other challenges.

A good laboratory will survey the literature and advise you of the feasibility of bench synthesis of a metabolite or impurity. If it does not appear to be accessible, they should offer other alternatives, such as forced degradation of parent material, isolation from *in vitro* and/or *in vivo* samples, or a growing arsenal of nontraditional methods such as microbial bioreactors or biomimetic oxidation.

## 9. OTHER ANALYTICAL SUPPORT

It is difficult to denote a single ultimate goal of any particular metabolite or impurity profiling exercise, but a particularly satisfying one is the unequivocal identification of what once may have been referred to in the Sponsor's laboratory as "Unknown X." If you are still at the "Unknown X" stage, but eventually intend to have the analyte synthesized, talk with your custom synthesis laboratory. A good laboratory will offer both synthesis services and trace analysis and structural chemistry support, so they can put a face and name to the unknown.

## 10. INTELLECTUAL PROPERTY

At its heart, the synthesis of new chemical entities is invention. There are few things more satisfying to a custom synthesis operation than being partners in your inventive process. Patents are extremely valuable business assets, worthy of protection and defense. The very best custom synthesis laboratories do more than deliver a product. They provide expert documentation of the synthesis, as well as characterization of intermediates and final product. Some can even help support IP claims by providing expert consultation or even testimony on API synthesis, impurity profiling and replication of synthetic processes.

## CONCLUSION

As noted above, many decisions must be made and much knowledge shared before embarking on a custom synthesis program. Viewing your synthesis provider as a development partner—rather than a vendor—is far more likely to end in a productive, long-term relationship.

In the end, you will want to make sure that your extraordinary needs find a match of extraordinary support—Technical Expertise, Timeliness, Quality, and a willingness to be a partner and not just a supplier.

## ABOUT EAG

EAG maintains four Class 10,000 synthesis suites and the rigorous CGMP-compliant environment required to produce certified radiolabeled product suitable for human clinical trials. Our team of developmental synthetic chemists is quite simply technically exceptional—and is backed by dedicated analytical and quality control scientific staff. No CRO has more experience supporting clinical studies using labeled isotopes--or takes greater pride in helping the pharmaceutical industry bring life-changing therapies to patients who need them.

Indeed, EAG scientists are proud to have partnered with companies involved in the new drug approval process and such partnerships have resulted in regulatory approvals for the stakeholders most affected in this process- the patients awaiting these life changing compounds.

## BIOGRAPHIES

**James Schmidt,** EAG Life Sciences Senior Scientific Advisor for Custom Synthesis, brings nearly three decades of experience in xenobiotic metabolism, bio-analytical chemistry, and structural elucidation with private, government, and industrial laboratories. His special interests include small molecule metabolic stability, metabolite profiling and identification, chiral separations, and pharmacologically active metabolites. He is the author or co-author of several peer-reviewed posters and papers, as well as innumerable scientific reports to satisfy regulatory requirements of both the EPA and FDA. Most recently, he contributed the chapter on "Metabolite Profiling" to the book New Horizons in Predictive Drug Metabolism and Pharmacokinetics (Alan G. E Wilson, ed., RSC Publishing, 2015). James is based at our Columbia, Missouri (formerly ABC Laboratories) location.

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