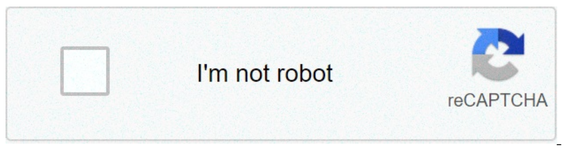

Continue

Solution phase peptide synthesis pdf

This website uses cookies. By continuing to use this website you are giving consent to cookies being used. For information on cookies and how you can disable them visit our Privacy and Cookie Policy. Got it, thanks! Biphalin analogs containing β(3)-homo-amino acids at the 4,4' positions: Synthesis and opioid activity profiles. Frączak O, Lasota A, Kosson P, Leśniak A, Muchowska A, Lipkowski AW, Olma A. Frączak O, et al. Peptides. 2015 Apr;66:13-8. doi: 10.1016/j.peptides.2015.02.004. Epub 2015 Feb 20. Peptides. 2015. PMID: 25708417 More and more groups are exploring the utility of peptides with an ever widening variety of applications. And although peptides are getting cheaper to purchase outright, many groups are continuing to bring peptide synthesis in house. As more groups join the peptide community, I frequently encounter questions about the basics of peptide synthesis. In today's post, I'd like to cover a little history of solid phase synthesis as well as highlight some differences between the chemistries. Chemistries and reagents for amide condensation reactions have existed for decades and been utilized to great effect by organic chemists globally. As interest in bioactive peptides grew during the mid 20th century though and along with it the need for synthetic analogues, it quickly became apparent that the "standard" solution phase strategies would not be sufficient. Solution phase peptide synthesis is typically very arduous and laborious - requiring long coupling reaction times and a need for recrystallization or column chromatography between each amino acid coupling. After making several analogues of bradykinin (9 amino acids), Bruce Merrifield sought an alternative and began developing methods for solid phase peptide synthesis, efforts for which he later won the Nobel Prize in Chemistry. The beauty of Merrifield's strategy is that chemical reagents can be reacted with a reactive moiety on a solid support, then removed by a simple filtration step, improving the throughput of peptide synthesis. Figure 1. General steps required to synthesize a peptide on solid phase. Protecting groups (PG) will be present on the amine nitrogen for iterative deprotection as well as any potentially reactive side chain groups. To implement a filtration-based strategy, Merrifield combined a functionalized, insoluble polymeric support and protecting group chemistry, enabling the iterative addition of each amino acid building block, Figure 1. Two types of protecting groups were required for this to be successful: 1) an amine-reactive reversible protecting group that could be liberated from the N-terminus and 2) protecting groups for the various polar and charged side chain moities that would be resistant to the frequent N-terminal deprotection conditions, yet sensitive to the conditions which liberate the final peptide sequence from the solid support. With these conditions in mind, Bruce worked to developed the strategy now known as the Boc synthesis strategy. In this strategy, the N-terminal amine is protected with a Boc group, Figure 2, which can then be removed with moderate concentrations (usually 25-50%) of trifluoracetic acid. Functional side chains are protected using chemical moities stable to the repeated TFA treatment, but can still be liberated during treatment with the resin cleavage cocktail. The final peptide is cleaved from the resin originally using hydrofluoric acid (HF). Other cleavage conditions have been identified due to the safety concerns associated with HF use. The principle in Boc chemistry is simple: protecting group and global cleavage from the solid support are removed based upon relative acid sensitivity with the amine nitrogen protecting group being the most sensitive (least acidic conditions) and global cleavage from the resin being the least sensitive (most acidic conditions). Figure 2. Mechanism for removal of a Boc protecting group from the N-terminus of a growing peptide chain using trifluoracetic acid (TFA). Several years after Merrifield's introduction of the Boc SPPS strategy and using chemistry pioneered by Louis Carpino, Atherton and Sheppard applied the Fmoc amine protecting group to solid phase peptide synthesis. Rather than take advantage of acidolysis sensitivity, Fmoc-based chemistry combines base sensitivity with acid sensitivity, Figure 3. Figure 3. Mechanism for removal of an Fmoc protecting group from the N-terminus of a growing peptide chain using piperidine. Fmoc-based SPPS has revolutionized synthetic accessibility to chemists in a number of ones. First, and possibly most importantly is that the chemical routinely employed in Fmoc chemistry exhibit a much greater safety profile than those used in Boc chemistry. Repeated handling of relatively concentrated TFA solutions and handling of HF has been eliminated. This improved safety profile has enabled inexperienced chemists to undertake peptide synthesis efforts with relative ease and success. But secondly, exploited acid and base lability has welcomed the introduction of a huge diversity of side chain protecting groups. Introduction of new, orthogonal protection strategies enables on-resin chemistries and modifications that would be much more difficult or even impossible with traditional Boc protocols. This becomes even more relevant when we consider the complexity of peptides that are now routinely synthesized using Fmoc protocols. Interested in performing peptide synthesis in your lab? Follow the link below to learn more about what you'll need to get started. Peptide synthesis is a complex and crucial process most commonly utilized in organic chemistry. During peptide synthesis, multiple amino acids are links by peptide bonds. Linking amino acids has been a common practice in organic chemistry for many years, but the process is evolving. Recent technological advancements and more specialized synthesis processes have enabled researchers to now synthesize natural peptides and generate unique peptides with increased accuracy and reliability. However, in order to answer the question, “what is peptide synthesis?”, one must dive a little deeper into the process’s intricacies and applications. What are peptides? One must first gain a base understanding of peptides to properly understand what peptide synthesis is. Peptides are comprised of short chains of amino acids, all of which are linked together by peptide bonds. Structurally speaking, peptides are quite similar to proteins, as they are both made up of amino acid chains which are held together via peptide bonds. The main distinguishing factor between peptides and proteins, however, is their size. Whereas proteins are generally made up of 50 or more amino acids, peptides are often on the smaller side, consisting of only two to 50 amino acids. Additionally, protein structures are slightly more complex and can adopt secondary, tertiary, and quaternary structures. Peptides offer less variation and are typically only divided into two subsets. Oligopeptides are those which possess fewer amino acids, typically between two and 20. Polypeptides are those which contain a higher number of amino acids and, when linked together, produce proteins. In essence, a protein is one large peptide comprised of many polypeptides. Peptide synthesis processes and procedures While peptides do occur naturally and can be found in all living organisms, synthetic peptide production is often used by researchers to produce specific peptides. In particular, researchers synthesize those that may be difficult to express in bacteria or to experiment with the incorporation of amino acids that are not typically found in peptides. The most common method for the synthetic peptide production is called solid-phase peptide synthesis. Solid-phase peptide synthesis First pioneered by American biochemist Robert Bruce Merrifield, solid-phase peptide synthesis has since become the leading method for the synthetic production of peptides. Solid-phase peptide synthesis, often abbreviated as SPPS, streamlines the process of synthetic peptide production by creating multiple successive amino acid reactions on a singular porous apparatus. Peptide synthesis occurs when the carboxyl group of an incoming amino acid is coupled to the N-terminus of an existing and growing peptide chain. Amino acids are added one at a time to the growing peptide chain in a precise, cyclic manner, taking special care to follow the necessary steps exactly. Analysts must be extremely cautious when creating this peptide chain, as amino acids possess multiple reactive groups and may cause side reactions which reduce the length of the chain or cause it to branch. In order to reduce the likelihood of these side reactions, the observing analyst must utilize protecting groups such as Fmoc or Boc. These base-labile and acid-labile protecting groups, respectively, help achieve chemoselectivity throughout the synthesis process, effectively reducing the likelihood of any unintended side reactions. Once the system has been treated with these temporary protecting groups—which can be added and removed with relative ease throughout the entire synthesis process—amino acids can be added to the peptide chain. Carbodiimides are used to activate the C-terminal carboxylic acid of the incoming amino acid, enabling it to link to the N-terminus of the growing peptide chain. As we’ve stated, the SPPS process is cyclical and these steps are repeated numerous times in order to achieve the desired peptide bond length. Once the peptide bond has reached the desired length it must be cleaved of any remaining protecting groups. Through a process called acidolysis, the peptide bond is stripped of its remaining protecting groups. That is to say, the N-terminal protecting group of the last-added amino acid, the C-terminal protecting group of the first amino acid, and any remaining side-chain protecting groups will be removed. The chemical chosen to complete this process will be dependent upon the protecting groups used in the system. Acid-based protecting groups, such as Boc, will require the use of strong acids, such as hydrogen bromide or hydrogen fluoride. Conversely, base-labile protecting groups, such as Fmoc, can be cleaved using a milder acid, such as trifluoroacetic acid (TFA). Because SPPS is such a complex procedure which requires extreme care and precision, it can also be quite time consuming. For this reason—among others—many laboratories turn to automated solid-phase extraction systems to streamline the process. These workstations enable the observing analyst to leave the system unattended while maintaining full confidence that the procedures will be completed with accuracy and efficiency. The workstations are able to accommodate larger samples and can be micro-controlled via computer to yield even higher precision and accuracy. For more information on how automated workstations can be utilized for solid-phase peptide synthesis, click here. Solution-phase peptide synthesis Another technique in the synthetic production of peptides is solution-phase peptide synthesis. Though less widely used, this technique utilizes many of the same procedures as SPPS. However, this process can be slightly more time consuming, as it requires the product peptide to be isolated from the solution after each reaction step. It is for this reason that solution-phase peptide synthesis has been replaced by SPPS in many laboratories. Solution-phase peptide synthesis is still often used in larger-scale production of peptides, however, which are typically used for industrial applications. Applications of peptide synthesis Peptide synthesis is often used in conjunction with epitope mapping and typically sees applications in medical sciences and biotechnology. Synthetic peptides are used to research potential cancer diagnoses and treatments and in the development of antibiotic drugs. When used in conjunction with epitope mapping—which is the process of identifying the bonding sites between an antibody and its antigen—peptide synthesis can be used to produce antibodies and create more intelligent vaccine designs. Synthetically produced peptides are also utilized in mass spectrometry-based applications, in which they can function as both standards and reagents. Last updated: May 8th, 2020 |Peptide bonds: Forming peptides from amino acids with the use of protecting groupsToday we’ll go deeper on how to synthesize the most important amides of all – peptides – with an important contribution from protecting group chemistry.Table of Contents1. What Are Peptide Bonds?A “peptide bond” is an amide linkage (see Amides: Properties. Synthesis, and Nomenclature) that connects two amino acids, as in the “dipeptides” L-phenylalanyl-L-valine (below left) and L-leucyl-L-alanine (below right):2. The “Proteinogenic” Amino AcidsProteinogenic amino acids are the building blocks of proteins. In addition to the 20 amino acids directly encoded by the genome, two other amino acids are coded into proteins under special circumstances: selenocysteine (present in eukaryotes, including humans) and pyrrolysine (found only in methane-producing bacteria).With the exception of (achiral) glycine, all proteinogenic amino acids are L-amino acids, where the “L-” prefix relates the stereochemistry of the amino acid relative to that of L-glyceraldehyde [See post: D and L Sugars] .Of the chiral amino acids, all are S, with the exception of cysteine and selenocysteine (because sulfur and selenium have a higher priority under the CIP system.)3. Synthesis of A Simple Dipeptide Without Protecting Groups (is not advisable!)Let’s build a simple dipeptide between two of these amino acids. For simplicity’s sake, we’ll pick two from the “hydrophobic sidechain” group, alanine (Ala) and leucine (Leu), since their sidechains don’t need additional protecting groups.What do we need to do to make L-Ala-L-Leu ?Surveying the methods previously covered to make amides, it might seem simple.Why not take 1 equivalent each of L-alanine and L-phenylalanine, add a coupling agent like N,N-dicyclohexylcarbodiimide (DCC) and patiently wait for our product to appear?What could possibly go wrong?Well, this will give us some of our desired product. But it won’t do so efficiently!That’s because each amino acid has two reactive termini – an amine and a carboxylic acid – and they can bond together in multiple ways.Just like the letters A and L can combine to make the words AL and LA, in addition to Ala-Leu (our desired product) we will also get Leu-Ala.Furthermore, since we’re not adding single molecules together but molar quantities (even a millionth of a mole (a “micromole”) has 1017 molecules in it) we also have the possibility of forming the “homo-dipeptides” AA (Ala-Ala) and LL (Leu-Leu).And that’s just a start. No matter how you slice it you’re looking at a low yield (95%) of a single product![Note: the Boc group is a popular carbamate protecting group for amines; “Boc” stands for t-butyloxycarbonyl]5. Synthesis of Longer Peptides – Tripeptides and TetrapeptidesThe good news is that we don’t have to stop at the dipeptide. If we choose protecting groups that can be removed selectively (and the carbamate / ester pair qualifies) then we can then deprotect the carbamate, and add a third amino acid.The choice of carbamate protecting group here was t-butoxycarbonyl (Boc) which is removed with strong acid (trifluoroacetic acid, abbreviated as TFA).Treatment with TFA removes the Boc group but leaves the methyl ester alone.So if we treat the dipeptide with TFA, we liberate the amine nitrogen, and can react with another Boc-protected amino acid in the presence of DCC to get a tripeptide.If we’re keen, we can even extend the same method to build a tetrapeptide, a pentapeptide… or beyond!It’s not unreasonable to consider this method for longer peptides.For instance, take something like bradykinin, a 9-peptide chain that causes dilation of blood vessels leading to a rapid drop in blood pressure. (Your body releases bradykinin in response to snake bites, which is how it was originally discovered.)It might be interesting to synthesize variants of bradykinin where some of the amino acids are swapped out for other ones. In order to do that, we’d need to be able to synthesize it.So how effective could it be? If each peptide coupling step has a yield of about 95%, then our overall yield for making bradykinin would be (0.95)9 , or 63%. That’s actually pretty good! A lot of chemists would be happy to get a yield of 63% for a single reaction, let me tell you.If the yields are high enough, one can even imagine building something crazy like insulin (51 peptide residues). That’s 7% yield for 51 steps.Is this possible?Yes… but it requires a clever modification that won its inventor, Bruce Merrifield, the 1984 Nobel Prize in Chemistry.6. Bonus Topic: Solid-Phase Peptide SynthesisWhat follows below is more supplemental than anything else, but given the importance of the topic, both interesting and useful.In 1963 a chemist at Rockefeller University named Bruce Merrifield published a paper that would revolutionize how peptides were synthesized, and eventually make the synthesis of long peptides routine.It was entitled: “Solid-Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide“.Here’s the key idea.Recall that in our original scheme (above) we protected the carboxylic acid as a methyl ester, which stays the same throughout the whole peptide synthesis.Merrifield’s idea was: what if we find a way to attach the carboxylic acid to a functional group that is itself linked to a polymer bead? Not only would this also protect the carboxylic acid, it would drastically improve the ease of separations.Why? Because instead of having to purify the final product by crystallization or column chromatography, you purify by filtering off the polymer beads (each 200-500 μm) and washing them to remove excess reagents.The polymer beads themselves are pretty small. A typical size is 200 micrometers. Each bead can load about 4 nanomoles of amino acid.[Brandon Finlay from ChemTips shows a picture here]The video posted below is not mine, but it gives you an idea of the process.At about 0:34 you can see how small the beads are.The starting point for the Merrifield process is crosslinked polystyrene. which behaves like one big interlinked molecule. Polystyrene is then attached to a linker, which usually terminates with an NH2 group. This itself is usually protected; in order to activate the linker, you need to remove the protecting group cap.The polymer bead needs to swell in a solvent in order for functional groups on the solid support to undergo reactions efficiently.The essential procedure is: swell –> add reagents –> wait –> filter –> wash, and repeat. Beads stay in the reaction vessel the whole time. There’s also usually some kind of capping step to make sure any unreacted amines don’t participate in the next reaction.It’s possible to make peptides up to about 50 units this way. In highly automated systems one can be even more ambitious.Merrifield started knocking off peptides in the 1960s. Bradykinin was made in 8 days and 68% overall yield. one example. Insulin was made two years later. The crowning achievement of this initial period was probably ribonuclease A, which has 150 amino acid residues.The original Merrifield process has been significantly modified and improved. Originally, removal of the linker required harsh conditions (strong acid). Today, procedures usually employ FMOC protecting groups instead of BOC, which allow for deprotection with mild amine base (piperidine). A galaxy of new resins, linkers, and coupling procedures have been subsequently developed. The Wikipedia article on solid-phase peptide synthesis is an OK place to start.NotesNote 1. Cysteine (and selenocysteine) are L, but R, because sulfur has a higher priority within the Cahn-Ingold-Prelog system.(Advanced) References and Further ReadingThis is a major topic, as the synthesis of peptides is a global billion-dollar industry.THE SYNTHESIS OF AN OCTAPEPTIDE AMIDE WITH THE HORMONAL ACTIVITY OF OXYTOCIN Vincent du Vigneaud, Charlotte Ressler, College John M. Swan, Carleton W. Roberts, Panayotis G. Katsoyannis, and Samuel Gordon Journal of the American Chemical Society 1953, 75 (19), 4879-4880 DOI: 1021/ja01115a553The Synthesis of Oxytocin Vincent du Vigneaud, Charlotte Ressler, John M. Swan, Carleton W. Roberts, and Panayotis G. Katsoyannis Journal of the American Chemical Society 1954, 76 (12), 3115-3121 DOI: 1021/ja01641a004A Method of Synthesis of Long Peptide Chains Using a Synthesis of Oxytocin as an Example Miklos Bodanszky and Vincent du Vigneaud Journal of the American Chemical Society 1959, 81 (21), 5688-5691 DOI: 1021/ja01530a040 In the first half of the 20th century, peptide synthesis was done using standard organic chemistry solution phase techniques. This is now known as LPPS (liquid-phase peptide synthesis). du Vigneaud received the Nobel Prize in chemistry in 1955 for his work in showing that peptide synthesis could be achieved, using the correct choice of protecting groups and synthetic strategies.In 1963, Prof. Robert Bruce Merrifield (Rockefeller U., New York) revolutionized peptide synthesis by coming up with the SPPS (Solid-Phase Peptide Synthesis) method, making the synthesis of long peptide chains much more feasible. The C-terminus is bound to a polymer resin, and the amino acids are added one at a time following the same cycle: deprotect, wash, couple the next amino acid (with a peptide-coupling reagent such as DCC), wash, deprotect the N-terminus again, and so on. Merrifield’s method came to be called the Boc/Bzl strategy due to the protecting groups employed (Boc for the nitrogen atoms, and Bzl (benzyl) for the side chains). The catch is that final cleavage of the peptide from the resin using this method requires anhydrous HF, which is not easy to handle.Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide R. B. Merrifield Journal of the American Chemical Society 1963, 85 (14), 2149-2154 DOI: 10.1021/ja00897a025 This is the paper that started it all – a single-author publication by Prof. Merrifield using the SPPS method to make a tetrapeptide. This remains one of the most highly-viewed and highly-cited papers in JACS, even today.The Synthesis of Bovine Insulin by the Solid Phase Method Marglin and R. B. Merrifield Journal of the American Chemical Society 1966, 88 (21), 5051-5052 DOI: 10.1021/ja00973a068 Insulin is a billion-dollar hormone as its dysregulation is what causes diabetes. This paper shows that insulin can be made through SPPS methods. Insulin is tricky to make as it has 2 chains (the A and B chain) connected through disulfide bonds. Interestingly, Merrifield synthesized the active hormone by combining both chains with protected thiols (protected as sulfonates), which he then reduced to the thiol, and then oxidized in air in a basic medium (pH 10). This is called undirected or air oxidation since the disulfide bonds are not being formed selectively; luckily they formed correctly in this case. Insulin today is not manufactured by the SPPS method due to these complications; instead it is made through a recombinant process.The Synthesis of Ribonuclease A Bernd Gutte and R. B. Merrifield Journal of Biological Chemistry 1971, 246 (6), 1922-1941 This is the crowning achievement of SPPS – the synthesis of a 124-mer peptide (or protein, at this point), RNAse A.The Solid Phase Synthesis of Ribonuclease A by Robert Bruce Merrifield Nicole Kresge, Robert D. Simoni and Robert L. Hill Journal of Biological Chemistry 2006, 281 (26), e21 A short biographical account of Prof. Merrifield’s life. This mentions that he developed the first prototype of an automated peptide synthesizer working in the basement of his house in 1965!Solid Phase Synthesis Nobel Lecture, December 8, 1984 by Bruce Merrifield Merrifield’s Nobel Lecture upon receiving the Nobel Prize in Chemistry in 1984. This describes his life, how he conceived of and developed the SPPS process, and all the breakthroughs it has enabled.9-Fluorenylmethoxycarbonyl function, a new base-sensitive amino-protecting group Louis A. Carpino and Grace Y. Han Journal of the American Chemical Society 1970, 92 (19), 5748-5749 DOI: 10.1021/ja00722a0439-Fluorenylmethoxycarbonyl amino-protecting group Louis A. Carpino and Grace Y. Han The Journal of Organic Chemistry 1972 37 (22), 3404-3409 DOI: 10.1021/jo00795a005 The discovery and development of the Fmoc (9-fluorenylmethoxycarbonyl) group as a protecting group for amines has also improved the practice of peptide synthesis and SPPS.A mild procedure for solid phase peptide synthesis: use of fluorenylmethoxycarbonylamino-acids Atherton, Hazel Fox, Diana Harkiss, C. J. Logan, R. C. Sheppard and B. J. Williams J. Chem. Soc. Chem. Comm. 1978, 537-539 DOI: 10.1039/C39780000537 This is the first paper describing what is now known as the Fmoc/tBu SPPS process, which has largely supplanted the original Boc/Bzl process developed by Prof. Merrifield. The advantages with Fmoc-SPPS are multifold, but the main ones are simplicity and orthogonality. Amine deprotection is done with a base (20% piperidine in DMF is sufficient to deprotect an Fmoc-amine), and final cleavage of the peptide from the resin can be done with TFA (trifluoroacetic acid), which is much easier to handle than HF.Advances in Fmoc solid-phase peptide synthesis Raymond Behrendt, Peter White, and John Offer Peptide Sci. 2016, 22, 4-27 DOI: 10.1002/psc.2836 A modern review on Fmoc-SPPS that describes how far we have come and some of the challenges that remain. For instance, aggregation of peptide chains on the resin is a major issue in Fmoc-SPPS when synthesizing especially hydrophobic peptides, and there are ways to deal with it, such as the introduction of ‘kinking’ residues, like Pro or the use of pseudoprolines, which will revert back to the desired amino acids when the peptide is cleaved with TFA. Native Chemical Ligation is also used nowadays for the synthesis of especially long peptides, like Merrifield’s RNAse A.