International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

A Review on Chemistry of Peptide Synthesis

Ms. Hina Umar Momin*, Prof. Santosh Waghmare, Dr. H. V. Kamble
Department of Pharmaceutical Chemistry[1], Department of Pharmaceutical Chemistry[2], Department of Pharmacology[3]
[1, 2, 3] Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata.

Submitted: 10-12-2021 Accepted: 25-12-2021

ABSTRACT: For the proteins that cannot be expressed exactly by cell expression technology (e.g., proteins with multiple posttranslational variations or poisonous proteins), chemical conflation is an important cover. Since the invention of solid phase synthetic styles by Merrifield in 1963, the number of exploration groups fastening on peptide conflation has grown exponentially. Still, the original step-by- step conflation had limitations the chastity of the final product dropped with the number of coupling way. After the development of Boc and Fmoc guarding groups, new amino acid guarding groups and new ways were introduced to give high quality and volume peptide products. Scrap condensation was a popular system for peptide product in the 1980s, but unfortunately the rate of racemization and response difficulties proved lower than ideal. Kent andco-workers revolutionized peptide coupling by introducing the chemo picky response of vulnerable peptides, called native chemical ligation. Also, high-molecular- weight proteins must be synthesized using two or further peptide ligation way, and succession peptide ligation is such an effective way. In this paper, we reviewed the development of chemical protein conflation, including solid- phase peptide conflation, chemical ligation, and succession chemical ligation.

Keywords: peptide, protein, native chemical ligation, peptide synthesis.

I. INTRODUCTION:

Proteins play a pivotal part in abecedarian physiological and biochemical functions of life. With the development in decoding genome sequences of numerous organisms, an horizonless number of new peptide and protein sequences are discovered at a rapid-fire rate. The structures, functions, and mechanisms of action of these sequences should be illustrated. These studies begin with the product of a particular protein. In general, three ways are used for protein product, videlicet, in vivo expression, cell-free protein conflation, and chemical conflation. Utmost studies on the structure and function of proteins are grounded on the recombinant DNA- grounded expression of proteins in genetically finagled cells. This important technology enables the product of a large quantum of native proteins and allows changes in amino acid sequences of proteins via point- directed mutagenesis for farther exploration. Although it's the most extensively habituated system to produce a protein of interest, it has several disadvantages. First, it has difficulty in over- expressing proteins that are poisonous to the cell, similar aspro- teases (1). Second, given that living cells are used for protein product, similar conflation systems are innately limited to the 20 genetically decoded amino acids. Indeed though some synthetic amino acids can be incorporated genetically in bacteria, incentive, and mammalian cells through a number of orthogonal tRNA/ synthetase dyads, these styles are presently not accessible by utmost experimenters (2 – 5). Cell-free protein expression is an indispensable cell- grounded system because it offers a simple and flexible system for the rapid-fire conflation of folded proteins, drastically reducing the time taken to transfigure from DNA sequence to functional protein (6). Cell-free protein product is achieved by combining a crude lysate from growing cells, which contains all the necessary enzymes and ministry for protein conflation (including recap and restatement), with the exogenous force of essential amino acids, nucleotides, mariners, and energy-generating factors and introducing exogenous dispatches including RNA (mRNA) or DNA as template into the system (7, 8). The cell-free system presents two egregious advantages over in vivo protein expression. One is that cell-free systems allow the effective objectification of synthetic or chemically modified amino acids into the expressed protein at asked positions during

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

restatement, thereby generating new motes for proteomic applica-tions (9 – 14). Still, numerous unnatural amino acids are simply inharmonious with ribosomal polypeptide conflation (15). The other is that cell-free systems can produce proteins that aren't physiologically permitted by the living cell —e.g., poisonous, proteolytically sensitive, or unstable proteins (16). Chemical conflation is playing an decreasingly important part in protein product, especially after Merrifield's invention of solid- phase peptide conflation (SPPS) in 1963 (17). Nonetheless, homogeneous long peptides are delicate to gain through SPPS. Still, chemical ligation, which needs the SPPS to produce the parts of protein, can break this problem. The biggest advantages of this technology are the free objectification of unnatural amino acids and conflation of proteins, which are poisonous to the living cell. In this paper, SPPS and styles of chemical ligation were reviewed.

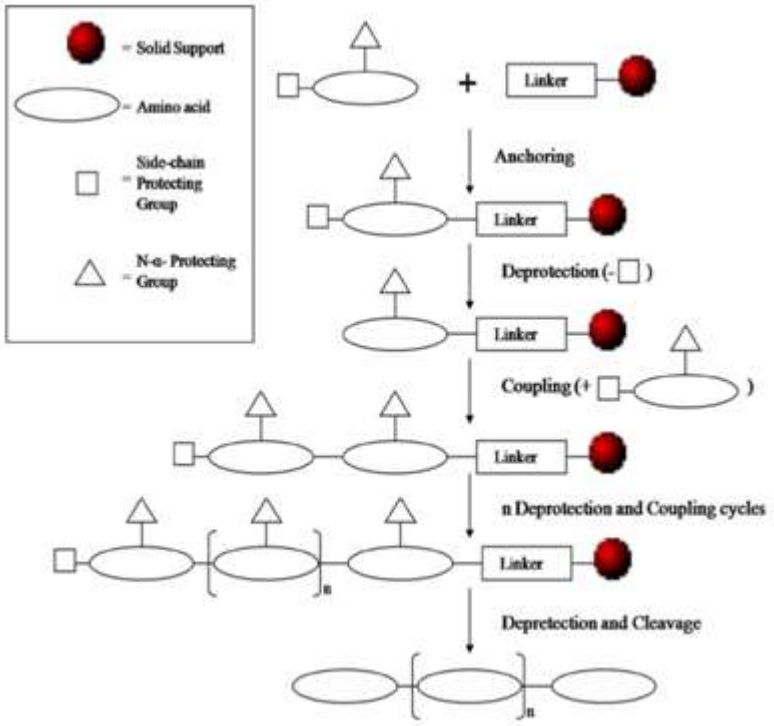
One approach to meet the demand for vim- drift- grounded pharmaceutical products like the mortal insulin and test pieces for the pharmaceutical exploration is the combinatorial chemistry. It was introduced in the 1980s, grounded on the pioneering workshop of Furka (18-21), Geysen (22-25), Houghten (26-29), Lam (30-33) and Moos (34-37). The combinatorial chemistry was con-sidered to be a promising technology and traces back to health-profitable questions raised in the 1930's (38-40). Until 1922 the quantum of insulin insulated from cattle pancreas satisfied the requirements of the diabetic dad-tient- centered care, but in the 1930s the difficulties in carrying sufficient quantities of pancreas organs in- crinkled putatively (41-43). The first chemical conflation of insulin, composed of 51 amino acids was published by Meierhofer and Zahn in 1963 (44). Before, the total chemical conflation of insulin wasn't assumed to be a successful specialized system and the interest for it remained solely academical. Semi conflation strategies also failed to secure reproducible yield rates (45-47) and a lack of available insulin passed. The dilemma was proved in the " National Diabetes Advisory Board " (1976), revealing the prognosticated demand of insulin until the time 2000, a task which natural sources fulfill (48).

SPPS

Prior to the development of SPPS, druggists performed peptide conflation via classical result- phase styles. Sanctification must be done after coupling every amino acid through birth and chromatography using a column of silica gel, which is time consuming and inconvenient. In 1963, Merrifield proposed an indispensable approach for preparing peptides (49). The principles of SPPS are illustrated inFig. 1. The N- defended C-terminal amino acid residue is anchored via its carboxyl group to a hydroxyl or amino resin to yield an ester-or amide- linked peptide that will eventually produce a C-terminal acid or a C-terminal amide peptide, independently. After loading the first amino acid, peptide conflation is stretched by the deprotection and coupling cycles from the C- boundary to the N- boundary (C? N strategy). Given that the removable guarding group in every cycle is guarding the amino group of the amino acid residue, the extension of peptide conflation is generally from the C-terminal to the N- outstation. After every step of deprotection or coupling, excess reagent is washed down by organic sol- reflections (e.g., DCM and DMF). All the functional groups on the side chains of the amino acids should be defended bysemi-permanent guarding groups, which avoid the side response with the reagents during the conflation. In general two strategies can be used for SPPS, videlicet, Boc and Fmoc approaches for thea-amino guarding group. Generally, Boc can be removed by 30 TFA in DCM and Fmoc can be removed by 20 piperidine in DMF. Final fractionalization of the peptidyl resin and side- chain deprotection requires a strong acid, similar as anhydrous hydrogen fluoride (HF) or trifluoromethanesulfonic acid (TFMSA), in the case of Boc chemistry, and TFA in Fmoc chemistry. The crude peptides can be anatomized and purified by HPLC and characterized by mass spectrometry.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

Fig. 1 Principle of SPPS



Kevin calls Leo and tells him to accelerate to the football game. When Leo arrives he sees Stargirl on the field cheering and doing strange capers each over the field. She's easily entertaining the crowd as they cheer her on. Still, the adjudicator isn't regaled. He orders her off. The police appear to be willing to remove her. But, also Stargirl gets the hint and runs off of the field as the followership cheers. Thousands of people show up for the coming game awaiting to see Stargirl cheer. But she does not show up. Several days latterly Mallory Stillwell, captain of the cheerleading team sits with Stargirl at lunch and invites her to come a cheerleader. Stargirl says yes. Stargirl seems to be getting further popular, and yet many want to really get close to her.

During the SPPS system (fig. 2), the resin is used as a support to which the growing peptide is anchored. First amino acid with temporary guarding groups on the reactive side chain and the nascence amino group (precluding polymerization) is attached to the resin via its C- boundary. After the addition of an amino acid, the protection group is removed and the resin washed previous to posterior additions. The process is repeated until the sequence is completed, whereupon the needed peptide is adhered from the resin (50). Boc and Fmoc guarding groups have frequently been used for side chain protection and are removed by trifluoroacetic acid or 20 piperidine in dimethylformamide, independently. Colorful resins have been used as a solid support in SPPS, for illustration polystyrene, Merrifield, hydroxymethyl, phenylacetamidomethyl, Wang and 4-methylbenzhydrylamine resins.

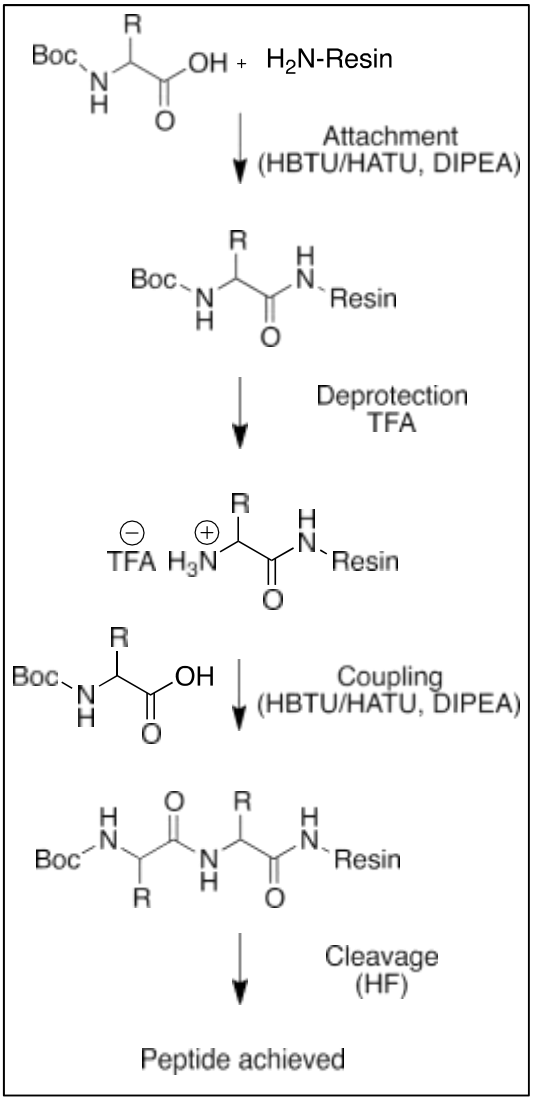
SPPS conflation was bettered by the development of fryer- supported SPPS especially when synthesizing long peptide sequences. Fryer irradiation allowed for the conflation of peptides in high yields and low degrees of racemization. Colorful peptides were synthesized in a shorter time when fryer-supported SPPS was applied rather of the traditional SPPS. Another advantage of fryer-supported SPPS use was the capability to control temperature and pressure while the conflation was in progress. General time and temperature conditions for fryer- supported SPPS using either Boc or Fmoc chemistry are listed below in Table 1. The limitations of this fashion can be the cost of resin and outfit.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

Table 1. In situ neutralization (optimized) protocol for microwave-SPPS [31,32].

Synthetic Cycle	Reagents	Time & Conditions
Deprotections	Trifluoroacetic acid (Boc chemistry)/ 20% piperidine in DMF (Fmoc chemistry)	1–5 min, 0 Watt, rt (Boc chemistry) or 70 °C (Fmoc chemistry)
Couplings	Amino acid, HBTU/HATU/HOBt/HOAt/DIC, DIPEA	5–15 min, 20 Watt, 50–70 °C

Fig 2. SPPS steps with Boc-chemistry.



International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

Chemical Ligations

Given that long peptides can not be fluently attained through SPPS, chemical ligation can help address this problem. First, small peptides are prepared by SPPS and also coupled via chemical ligation (Fig. 3).

Classical result synthetic chemistry uses completely defended peptide parts for condensation in organic sol- reflections (51). This approach to the conflation of long polypeptide chains suffers from a number of failings (52). First, the activation of the C-terminal carboxyl group of a defended peptide chain gives rise to epimerization of the C-terminal amino acid under introductory conditions for condensation with another peptide member nucleophile. Second, completely defended peptide parts are delicate to effectively purify and characterize. For illustration, electro- spray mass spectrometry has come one of the most useful tools for determining the covalent structure of vim- drift; this important system involves direct ionization of an analyte from waterless result. The effectiveness of similar ionization depends on the presence of multiple ionizable groups in the patch under study. The lack of similar groups in completely defended peptides precludes direct analysis by electrospray mass spectrometry. Third, and utmost seri-ously, numerous completely defended peptides have only limited solubility in organic detergents that are used for peptide conflation. The low attention of replying peptide parts frequently lead to slow and deficient coupling responses (53, 54).

The system developed by Blake and Li (33) (seeFig. 4) is a useful member condensation system because it involves the use of a unique C-terminal thiocarboxyl group for coupling with an N-terminal amine. The condensation between two peptides is achieved through picky activation of the thiocarboxyl group by tableware ions. (Gly17)-b-endorphin, b-lipotropin,a-inhibin-96, mortal pancreatic growth hormone- releasing factor, and two elision analogs (55) were successfully synthesized using this strategy.

Fig.3 Principle of chemical ligation

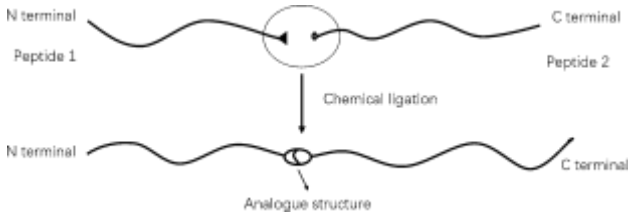


Fig. 4 Principle of Chemical Ligation

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

An indispensable thioester- grounded system can be employed to replace the thiocarboxylate, which is unsta-ble and gradationally decomposes by oxidative hydrolysis (). A thioester can also be actuated in the presence of tableware ions. The activation of thiocarboxylate or thioester by tableware ions is specific and functions in the presence of other free carboxyl groups on the amino acid side chains. Still, these condensation styles still bear par-tially defended peptides as structure blocks, and the effi-ciency of the intermolecular coupling response is innately low for large peptide parts because of the difficulty of achieving high molar attention for high-molecular- weight composites (58). Nonetheless, if the original con-centration of the replying carboxyl and amino groups can be increased by bringing the two peptides together, the propinquity effect may grease the peptide bond conformation response.

Application of Peptides and Proteins in Medicinal Chemistry.

Peptides are generally picky and efficient, acting on their targets in low attention, therefore are one of the stylish campaigners for medicine development and delivery. The Food and Drug Administration (FDA) has approved numerous peptide and peptide- grounded medicines for use as rectifiers (59-61). Presently, there are further than 60 peptide medicines on the request and over 500 peptides are in colorful stages of preclinical and clinical development (62). Peptide medicines have operations in the medical and pharmaceutical assiduity, especially for treatment of cancer (18), and metabolic diseases (17, including diabetes, rotundity, osteoporosis), and other medical conditions similar as mislike, immunological diseases, and cardiovascular complaint (63). In cancer exploration, Wilm's tumour-1 peptide in dendritice cell- grounded vaccines was plant to appreciatively impact the survival of cases withnon-small cell lung cancers (64). Another possible remedial target, insulin B chain peptide honored by a specific T cell receptor, was plant to be responsible for inauguration of diabetes (65). B- Type natriuretic peptide produced by cardiomycetes was used clinically and represents an efficient remedial strategy to treat mortal heart failure conditions (66). Short peptides lately reported by Dawgul etal. were plant to be active against Staphylococcus aureus infections therefore could be used to treat staphylococcal skin complaint (67).

Proteins are another clinically and commercially important class of rectifiers. Streptokinase is a protein medicine, which is currently available on the request for treatment of heart conditions, thrombosis, and embolism. Mutations of an adipocyte-specific buried protein leptin were shown to drop appetite and increase the metabolic rate in humans. This inheritable substantiation of leptin being an important controller of energy balance could be a way to treat rotundity (68). Capsid proteins from four mortal papillomavirus strains were successfully employed for vaccine development and retailed as forestallment against cervical cancer (69).

Peptide Manufacture

Chemical conflation, especially SPPS grounded on Fmoc chemistry, is currently the most popular choice of manufacturing procedure for peptides. This is because the solid phase manufacturing cost dropped (due to the lower cost of raw accoutrements, and frugality of scale), and the specialized advancements in chromatographic outfit and media. SPPS styles are briskly, more flexible (indesign of analogs) and less precious (bear lower process development, use general chemical and sanctification processes) for peptide manufacturing at over to a multi - 100 - kg scale than the use of recombinant technology. Still, there are peptide rectifiers (mortal glucagon, salmon calcitonin,etc.), which are manufactured using recombinant technology and it's anticipated that the operation of recombinant technology for peptide product will increase substantially due to its profitable possibility for scale up. Some peptides, like an HIV emulsion asset (36 amino acids; fuzeon) or a direct thrombin asset (20 amino acids; bivalirudin), are routinely manufactured on a large scale that exceeds 100 kg per time. To further ameliorate cell penetration, stability, particularity and targeting of peptides, the hydrocarbon stapling fashion was developed by Verdine etal. (70). The bioactive nascence spiral pack was introduced by the point-specific addition of a chemical brace. This system allowed the conformation of stable nascence spiral peptide structures important in numerous natural pathways, and has been used in direct, cyclic, and nanoparticle- grounded forms of peptides. Since also, several companies (e.g., AnaSpec, Aileron Rectifiers) have applied this fashion to enhance the pharmacological performance of remedial peptides.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 949-958 www.ijprajournal.com ISSN: 2249-7781

II. CONCLUSIONS

Chemical synthesis of a peptide or protein is crucial to analyze the structure and function of a protein. We reviewed most of the methodologies of chemical synthesis of proteins in this study. The successful application of peptide or protein drugs would drive the field of chemical protein synthesis to a broad space. All techniques described herein can be tailored to prepare a variety of peptides and proteins. Several peptide drugs have been already approved by FDA and have reached the market, which demonstrates the potential of peptides to be used as effective drugs.

ACKNOWLEDGMENTS:

We would like to acknowledge the Australian Research Council for their support of this work with the Professorial Research Fellowship to Istvan Toth (DP110100212) and an Australian Postdoctoral Fellowship to Pavla Simerska (DP1092829). This review was supported by the National Natural Science Foundation of China (No. 81400680, No. 31500723), the National Science Foundation of Tianjin (No. 17JCQNJC12800), Tianjin Science and Technology Plan Project (No. 14RCGFSY00147), and International S&T Cooperation Program of China (No. 2015DFG31850).

REFERENCES:

[1]. Dawson PE, Kent SBH (2000) Synthesis of native proteins by chemical ligation. Annu Rev Biochem 69:923–960
[2]. Wang L, Schultz PG (2005) Expanding the genetic code. AngewChem Int Ed 44(1):34–66
[3]. Anderson JC, Schultz PG (2003) Adaptation of an orthogonal archaeal leucyl-tRNA and synthetase pair for four-base, amber and opal suppression. Biochemistry 42(32):9598–9608
[4]. Edwards H, Schimmel P (1990) A bacterial amber suppressor in Saccharomyces cerevisiae is selectively recognized by a bacte- rial aminoacyl-tRNA synthetase. Mol Cell Biol 10(4):1633–1641
[5]. Sakamoto K, Hayashi A, Sakamoto A et al (2002) Site-specific incorporation of an unnatural amino acid into proteins in mammalian cells. Nucleic Acids Res 30(21):4692–4699
[6]. Murthy TVS, Wu WL, Qiu QQ et al (2004) Bacterial cell-free system for high-throughput protein expression and a compara- tive analysis of Escherichia coli cell-free and whole cell expression systems. Protein Expr Purif 36(2):217–225
[7]. Hoffmann M, Nemetz C, Madin K et al (2004) Rapid translationsystem: a novel cell-free way from gene to protein. Biotechnol Annu Rev 10:1–30
[8]. Jackson AM, Boutell J, Cooley N et al (2004) Cell-free protein synthesis for proteomics. Brief Funct Genom Proteom 2(4):308–319
[9]. Nemoto N, Miyamoto-Sato E, Yanagawa H (1999) Fluores- cence labeling of the C-terminus of proteins with a puromycin analogue in cell-free translation systems. FEBS Lett 462(1/ 2):43–46
[10]. Nemoto N, Miyamoto-Sato E, Husimi Y et al (1997) In vitro virus: bonding of mRNA bearing puromycin at the 3^0-terminal end to the C-terminal end of its encoded protein on the ribosome in vitro. FEBS Lett 414(2):405–408
[11]. Kohrer C, Xie L, Kellerer S et al (2001) Import of amber and ochre suppressor tRNAs into mammalian cells: a general approach to site-specific insertion of amino acid analogues into proteins. Proc Natl Acad Sci USA 98(25):14310–14315
[12]. Furka A. Study on the possibilities of systematic searching for pharmaceutically useful peptides. Notarized Report (File number 36237/1982, in Hungarian). 1982.
[13]. Sebestyen F, Dibo G, Kovacs A, Furka A. Chemical Synthesis of Peptide Libraries. Bioorganic & Medicinal Chemistry Letters. 1993; 3: 413-8.
[14]. Furka A. History of Combinatorial Chemistry. Drug Development Research. 1995; 36: 1-12.
[15]. Furka A. Combinatorial chemistry: 20 years on. Drug Discovery Today. 2002; 7: 1-4.
[16]. Geysen HM, Wagner CD, Bodnar WM, Markworth CJ, Parke GJ, Schoenen FJ, Wagner DS, Kinder DS. Isotope or mass encoding of combinatorial libraries. Chemistry & Biology. 1996; 3: 679-88.
[17]. Geysen HM. Combinatorial chemistry - past, present and future. Annals of Oncology. 1998; 9: 2.
[18]. Geysen HM, Schoenen F, Wagner D, Wagner R. Combinatorial compound libraries for drug discovery: An ongoing challenge. Nature Reviews Drug

Discovery. 2003; 2: 222-30.
[19]. Fitzgerald SH, Sabat M, Geysen M. Survey of the diversity space coverage of reported combinatorial libraries. Journal of Combinatorial Chemistry. 2007; 9: 724-34.
[20]. Blondelle SE, PerezPaya E, Dooley CT, Pinilla C, Houghten RA. Soluble Combinatorial Libraries of Organic, Peptidomimetic and Peptide Diversities. Trac-Trends in Analytical Chemistry. 1995; 14: 83-92.
[21]. Houghten RA. Parallel array and mixture-based synthetic combinatorial chemistry: Tools for the next millennium. Annual Review of Pharmacology and Toxicology. 2000; 40: 273-82.
[22]. Nefzi A, Ostresh JM, Yu JP, Houghten RA. Combinatorial chemistry: Libraries from libraries, the art of the diversity-oriented transformation of resin-bound peptides and chiral polyamides to low molecular weight acyclic and heterocyclic compounds. Journal of Organic Chemistry. 2004; 69: 3603-9.
[23]. Lopez-Vallejo F, Caulfield T, Martinez-Mayorga K, Giulianotti MA, Nefzi A, Houghten RA, Medina-Franco JL. Integrating Virtual Screening and Combinatorial Chemistry for Accelerated Drug Discovery. Combinatorial Chemistry & High Throughput Screening. 2011; 14: 475-87.
[24]. Salmon SE, Lam KS, Felder S, Yeoman H, Schlessinger J, Ullrich A, Krchnak V, Lebl M. Use of Large Combinatorial Chemical Libraries for Anticancer Drug Discovery. International Journal of Pharmacognosy. 1995; 33: 67-74.
[25]. Hruby VJ, Shenderovich M, Lam KS, Lebl M. Design considerations and computer modeling related to the development of molecular scaffolds and peptide mimetics for combinatorial chemistry. Molecular Diversity. 1996; 2: 46-56.
[26]. Aina OH, Sroka TC, Chen ML, Lam KS. Therapeutic cancer targeting peptides. Biopolymers. 2002; 66: 184-99.
[27]. Lam KS, Renil M. From combinatorial chemistry to chemical microarray. Current Opinion in Chemical Biology. 2002; 6: 353-8.
[28]. Lam KS. New aspects of natural products in drug discovery. Trends in Microbiology. 2007; 15: 279-89.
[29]. Moos WH, Hurt CR, Morales GA. Combinatorial chemistry: oh what a decade or two can do. Molecular Diversity. 2009; 13: 241-5.
[30]. 30. Desai MC, Zuckermann RN, Moos WH. Recent Advances in the Generation of Chemical Diversity Libraries. Drug Development Research. 1994; 33: 174-88.
[31]. 31. Moos WH. Combinatorial chemistry at cross roads. Abstracts of Papers of the American Chemical Society. 1997; 213: 386-ORGN.
[32]. 32. Merrifield RB (1963) Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. J Am Chem Soc 85(14):2149–2154.
[33]. 33. Albericio F (2000) Solid-phase synthesis: a practical guide. CRC Press, Boca Raton Ng S, Goodson B, Ehrhardt A, Moos WH, Siani M, Winter J. Combinatorial discovery process yields antimicrobial peptoids. Bioorganic & Medicinal Chemistry. 1999; 7: 1781-5.
[34]. 34. Messeguer A, Cortes N. Combinatorial chemistry in cancer research. Clin Transl Oncol. 2007; 9: 83-92.
[35]. 35. Diller DJ. The synergy between combinatorial chemistry and high-throughput screening. Curr Opin Drug Discov Devel. 2008; 11: 346-55.
[36]. 36. McGee JE, Roy A. Patent review. Comb Chem High Throughput Screen. 2011; 14: 926-8.
[37]. Oprea TI, Davis AM, Teague SJ, Leeson PD. Is there a difference between leads and drugs? A historical perspective. J Chem Inf Comput Sci. 2001; 41: 1308-15.
[38]. 37. Kvetensky J. [Historical evolution of knowledge of diabetes mellitus before the discovery of insulin. Review]. Vnitr Lek. 1972; 18: 67-72.
[39]. 38. Martin FI. The nature and use of purified insulins. Aust Fam Physician. 1976; 5: 514-25.
[40]. Bliss M. The history of insulin. Diabetes Care. 1993; 16 Suppl 3: 4-7.
[41]. Meienhofer J, Schnabel E, Bremer H, Brinkhoff O, Zabel R, Sroka W, Klostermayer H, Brandenburg D, Okuda T, Zahn H. [Synthesis of insulin chains and their combination to insulin-active preparations]. Z Naturforsch B. 1963; 18: 1120-1.
[42]. Ruttenberg MA. Human insulin: facile

synthesis by modification of porcine insulin. Science. 1972; 177: 623-6.
[43]. Geiger R, Obermeier R. Insulin synthesis from natural chains by means of reversible bridging compounds. Biochem Biophys Res Commun. 1973; 55: 60-6.
[44]. Obermeier R, Geiger R. A new semisynthesis of human insulin. Hoppe Seylers Z Physiol Chem. 1976; 357: 759-67.
[45]. Obermeier R, Seipke G. Enzyme-Catalyzed Semisyntheses with Porcine Insulin. Process Biochemistry. 1984; 19: 29-32.
[46]. United States.Congress.Senate.Committee on Labor and Public Welfare.Subcommittee on Health. National Diabetes Advisory Board act 1976. Washington: University of Michigan Library. 1976: 1-116.
[47]. Aimoto S (2001) Contemporary methods for peptide and protein synthesis. Curr Org Chem 5(1):45–87.
[48]. Hojo H, Aimoto S (1991) Polypeptide synthesis using the S-alkyl thioester of a partially protected peptide segment. Synthesis of the DNA-binding domain of c-Myb protein (142–193)-NH_2. Bull Chem Soc Jpn 64(1):111–117
[49]. Liu CF, Tam JP (1994) Chemical ligation approach to form a peptide bond between unprotected peptide segments. Concept and model study. J Am Chem Soc 116(10):4149–4153
[50]. Hughes, B. 2009 fda drug approvals. Nat. Rev. Drug. Discov. **2010**, 9, 89–92.
[51]. Mullard, A. 2010 fda drug approvals. Nat. Rev. Drug. Discov. **2011**, 10, 82–85.
[52]. Mullard, A. 2011 fda drug approvals. Nat. Rev. Drug. Discov. **2012**, 11, 91–94.
[53]. Mullard, A. 2012 fda drug approvals. Nat. Rev. Drug. Discov. **2013**, 12, 87–90.
[54]. Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. Synthetic therapeutic peptides: Science and market. Drug Discov. Today **2010**, 15, 40–56.
[55]. Ayoub, M.; Scheidegger, D. Peptide drugs, overcoming the challenges, a growing business.
[56]. Chim. Oggi. **2006**, 24, 46–48.
[57]. Takahashi, H.; Okamoto, M.; Shimodaira, S.; Tsujitani, S.-I.; Nagaya, M.; Ishidao, T.; Kishimoto, J.; Yonemitsu, Y. Impact of dendritic cell vaccines pulsed with wilms' tumour-1 peptide antigen on the survival of patients with advanced non-small cell lung cancers. Eur. J. Cancer **2012**, **49**, 852–859.
[58]. Sosinowski, T.; Eisenbarth, G.S. Type 1 diabetes: Primary antigen/peptide/register/trimolecular complex. Immunol. Res. **2012**, 55, 270–276.
[59]. Chen, H.H.; Glockner, J.F.; Schirger, J.A.; Cataliotti, A.; Redfield, M.M.; Burnett, J.C. Novel protein therapeutics for systolic heart failure: chronic subcutaneous B-type natruiuretic peptide. J. Am. Coll. Cardiol. **2012**, 60, 2305–2312.
[60]. Dawgul, M.; Baranska-Rybak, W.; Kamysz, E.; Karafova, A.; Nowicki, R.; Kamysz, W. Activity of short lipopeptides and conventional antimicrobials against planktonic cells and biofilms formed by clinical strains of staphylococcus aureus. Future **2012**, 4, 1541–1551.
[61]. Coles, D.J.; Simerska, P.; Fujita, Y.; Toth, I. The influence of incorporating lipids or liposaccharides on the particle size of peptide therapeutics. Biopolymers Pept. Sci. **2011**, 96, 172–176.
[62]. Seong BL, Lee CP, RajBhandary UL (1989) Suppression of amber codons in vivo as evidence that mutants derived from Escherichia coli initiator tRNA can act at the step of elongation in protein synthesis. J Biol Chem 264(11):6504–6508
[63]. Mamaev S, Olejnik J, Olejnik EK et al (2004) Cell-free N-ter- minal protein labeling using initiator suppressor tRNA. Anal Biochem 326(1):25–32
[64]. Olejnik J, Gite S, Mamaev S et al (2005) N-terminal labeling of proteins using initiator tRNA. Methods 36(3):252–260
[65]. Cornish VW, Mendel D, Schultz PG (1995) Probing protein structure and function with an expanded genetic code. Angew Chem Int Ed 34(6):621–633
[66]. Sakanyan V (2005) High-throughput and multiplexed protein array technology: protein–DNA and protein–protein interactions. J Chromatogr B Anal Technol Biomed Life Sci 815(1/ 2):77–95
[67]. Merrifield RB (1963) Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. J Am Chem Soc 85(14):2149–2154
[68]. Channabasavaiah K, Stewart JM (1979) New analogs of luli- berin which inhibit ovulation in the rat. Biochem Biophys Res Commun 86(4):1266–1273
[69]. Schafmeister, C.E.; Po, J.; Verdine, G.L. An

all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. J. Am. Chem. Soc. **2000**, 122, 5891–5892.

[70]. Verdine, G.L.; Hilinski, G. Stapled peptides for intracellular drug targets. Methods Enzymol.

[71]. **2012**, 503, 3–33.

[72]. Walensky, L.D.; Kung, A.L.; Escher, I.; Malia, T.J.; Barbuto, S.; Wright, R.D.; Wagner, G.; Verdine, G.L.; Korsmeyer, S.J. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Sci. Signal. **2004**, 305, 1466–1470.

[73]. Rojas-Cervellera, V.c.; Giralt, E.; Rovira, C. Staple motifs, initial steps in the formation of thiolate-protected gold nanoparticles: How do they form? Inorg. Chem. **2012**, 51, 11422–11429.

[74]. Green, B.R.; Klein, B.D.; Lee, H.-K.; Smith, M.D.; Steve White, H.; Bulaj, G. Cyclic analogs of galanin and neuropeptide Y by hydrocarbon stapling. Bioorg. Med. Chem. **2012**, 21, 303–310.