Peptides have a broad number of applications: from biochemical tools to diagnostic kits; from nanotechnology to new materials; and from vaccines to drugs. Increased interest in peptides in all these fields is exemplified by the growing number of peptide-based drugs available [1,2]. Table 1 shows the peptide drugs accepted by the US Food and Drug Administration (FDA) between Jan 2015 and May 2021 [3,4]. During these six and a half years, 21 peptide-based drugs have been launched onto the market. This number accounts for approximately 5% of the total number of drugs, including both new chemical entities and biologics, approved by the FDA in the same period. Importantly, although cancer and metabolism continue to be the main target, peptides are being used for a much wider spectrum of indications and they continue to gain applications with time (Table 1).

| Year | Active Ingredient | Trade Name | Indication | Characteristics |
|------|------------------|------------|------------|-----------------|
| 2015 | Insulin degludec  | Tresiba®   | Diabetes   | Modified insulin with an aa deletion and a hexadecanedioic acid via γ-Glu at Lys (B29) |
| 2015 | Ixazomib         | Ninlar®    | Multiple myeloma | N-acylated, C-boronic acid dipeptide |
| 2016 | Adlyxin          | Lixisenatide® | Diabetes | 44 aa GLP-1 peptide with Lys6 at the C-terminal |
| 2017 | Abaloparatide    | Tymlos®    | Osteoporosis | 34 aa analog of parathyroid hormone-related protein |
| 2017 | Angiotensin II   | Giapreza®  | Hypotension | Natural octapeptide |
| 2017 | Etecalcitide     | Parsabiv®  | Hyperparathyroidism | 8 aa in two Cys chains: Ac-DCys-DAla-(DArg)3-DAla-DArg-NH2-L-Cys |
| 2017 | Macimorelin      | Macrilen®  | Growth hormone deficiency | Pseudotripeptide N-formylated |
Table 1. Cont.

| Year | Active Ingredient | Trade Name | Indication | Characteristics |
|------|------------------|------------|------------|-----------------|
| 2017 | Plecanatide      | Trulance®  | Chronic idiopathic constipation | 16 aa with two disulfides |
| 2017 | Semaglutide      | Ozempic®   | Diabetes   | GLP-1 peptide (31 aa in the chain) with hexadecanedioic acid via γ-Glu and mini PEG at Lys |
| 2018 | 177Lu DOTA-TATE  | Lutathera® | Neuroendocrine tumors, theranostics | 177Lu chelated by DOTA bound to Tyr3-octreotate |
| 2019 | Afamelanotide    | Scennesse® | Skin damage and pain | 13 aa linear peptide analog of α-MSH |
| 2019 | Bremelanotide    | Vyleesi®   | Hypoactive sexual desire in women | 7 aa cyclic peptide analog of α-MSH |
| 2019 | Enfortumab       | PADCEV®    | Cancers expressing nectin-4 | ADC with a synthetic analog of the marine natural peptide dolastatin 10 (5 residues) |
| 2019 | 68Ga DOTA-TOC    |           | Neuroendocrine tumors, diagnostics | 68Ga chelated by DOTA bound to Tyr3-octreotate |
| 2019 | Polatuzumab      | Vedotin-PiQ Polivy® | Diffuse large B-cell lymphoma | ADC with a synthetic analog of dolastatin 10 (5 residues) |
| 2020 | Setmelanotide    | Imcivree®  | Obesity    | 8 aa, cyclic disulfide |
| 2020 | 64Cu-DOTATATE    | Detectnet® | Scintigraphic imaging | 64Cu chelated by DOTA bound to Tyr3-octreotate |
| 2020 | 68Ga-PSMA-11     |            | Diagnosis of recurrent prostate carcinoma | 68Ga chelated by HBED-CC with a pending urea-based peptidomimetic |
| 2020 | Belantamab       | Blenrep®   | Relapsed or refractory multiple myeloma | ADC with a synthetic analog of the marine natural peptide dolastatin 10 (5 residues) |
| 2021 | Voclosporin      | Lupkynis®  | Lupus nephritis | Analog of cyclosporin, 11 aa cyclic peptide with several N-methyl aa |
| 2021 | Dasiglucagon     | Zegalogue® | Hypoglycemia in individuals with diabetes | 29 aa linear peptide |

Only a few decades ago, it was unthinkable to consider producing an active pharmaceuti-
cal ingredient (API) of a chemical entity which requires more than seventy chemical steps—as in the case of Fuzeon® (enfurtivide, T20) [5]—fulfilling the requirements of the regulatory agencies, targeting both purity of the peptide API and identification of the impurities. This has been possible thanks to the breakthroughs in three fields: (i) chemical synthesis; (ii) purification; and (iii) characterization, which allowed consideration of peptides, and oligonucleotides too, as small molecules from purity and characterization points of view.

Coincidentally, at the same time, in the late fifties and early sixties, in the USA East Coast, Nobel Laureate R. Bruce Merrifield (Rockefeller University, New York) and the entrepreneur Jim Waters (Water Corporation, Milford, Massachusetts, USA) developed two techniques, for synthesis and purification, respectively, that over time proved to be crucial in making peptides and oligonucleotides (TIDES) an alternative to small molecules and biologics as drugs. Interestingly, the two techniques were based on a similar concept, namely the concourse of a solid support—a resin.
Merrifield first envisaged and then implemented a solid polymeric protecting group for the C-terminal carboxylic group, in the so-called solid-phase peptide synthesis (SPPS) methodology [6]. Thus, the growing peptide chain can be elongated with N-protected amino acids, while the peptide chain is in a pseudo-soluble state anchored to the solid support (resin). Thus, all reactions—coupling of the incoming residue and removal of the N-protecting group—can be carried out using excesses of reagents, thus ensuring excellent yields. Intermediates are not isolated or characterized, and the excesses of reagents and soluble side products are removed by filtration and extensive washing with solvents. Once the sequence is completed, the unprotected peptide is cleaved from the resin. In recent years, resins, linkers, protecting groups, coupling reagents, solvents, and finally green approaches have been described for fine tuning the SPPS approach.

Reversed-phase high-performance liquid chromatography (RP-HPLC), whose roots are found in the pioneering work of Waters [7], is based on the hydrophobic interaction between the molecules—peptides in this case—to be purified in a hydrophilic mobile phase, and the hydrophobic moieties (long alkyl chains and others), which are attached to the solid support, the stationary phase. Jean Rivier linked SPPS and RP-HPLC [8]. Thus, using an analog of LH-RH as a peptide model, he showed that the synthesis of a peptide at industrial scale can be carried out using SPPS techniques, rendering a crude product with sufficient quality to be purified by RH-HPLC.

Mass spectrometry (MS) is probably the technique that has evolved most over time [9]. In combination with HPLC (separation by HPLC and detection by MS), it has become a key tool through which to detect and, more importantly, identify the chemical structure of the impurities present after synthesis. These impurities often have subtle structural changes that hinder their separation and identification. In this regard, they can contain one amino acid less or one more, deletion or double hit incorporation, respectively; they can be an epimer (racemization of just one residue) or a β-peptide (isomerization of Ser- or Asp); and the residues of Cys, Met, and Trp are able to undergo oxidation, or those of Cys, Met, Trp, and Tyr alkylation too [10].

The list of peptides that have reached the market (Table 1) supports the strength and the importance of these compounds in the pharmaceutical arena. In this context, we have decided to publish a Special Issue in *Applied Sciences*, entitled “Latest Advances on Synthesis, Purification, and Characterization of Peptides and their Applications”, which provides excellent reviews and quality research articles covering the most recent developments in peptide synthesis, purification, and analysis. It is hoped that some of these new synthetic “tricks” will soon form part of the toolbox for the synthesis of peptides of therapeutic interest.

**Author Contributions:** A.E.-F., B.G.d.I.T. and F.A. equally contributed to writing the article. All authors have read and agreed to the published version of the manuscript.

**Funding:** The work in the laboratory of the authors was funded in part by the following: National Research Foundation (NRF) (Blue Skies Research Program # 120386).

**Conflicts of Interest:** The authors declare no conflict of interest.

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