

Neuland and Jitsubo collaborate for Peptide Synthesis

March 5, 2018 - Jitsubo Co., Ltd. (Head Office: Yokohama, Kanagawa) and Neuland Laboratories Limited (an India-based CMO) announced that we have entered into a strategic collaboration in peptide research.

Jitsubo's patented **Molecular Hiving™** is a technology to manufacture peptides with innovative TAG-assisted liquid phase peptide synthesis (LPPS) and provides us with a high-quality and low-cost peptide APIs with short lead time.

Neuland's patented peptide column purification technology using **NeuCoat™** as stationary phase enables higher sample loading during (7 to 10X with respect to conventional preparative RP-HPLC).

Neuland and Jitsubo are jointly developing peptide APIs employing the **Molecular Hiving** technology and Neuland's proprietary purification technology (**NeuCoat**). We aim at contributing to the global healthcare industry by providing high-quality but affordable peptides to the market.

Neuland Laboratories

Neuland is a publicly listed company headquartered in India, with offices in the US and Japan. Neuland has three major business verticals – API, CMS and Peptides and has been producing quality APIs for 34 years with a customer-base across 80+ countries. Neuland has expertise in solution, solid-phase and hybrid synthesis methodologies for Peptides and has a proprietary preparative HPLC technique which yields 10-20X better throughput. Neuland has a stellar track record of successful regulatory inspections from USFDA (inspected 12 times), PMDA-Japan, EDQM, EMA, KFDA, TGA, CFDA, COFEPRIS (Mexico), ANVISA (Brazil) and AFSSAPS (France).

2x 250L Solution Phase Reactors
100L Solid Phase Reactors
Nutsche filter SS316, 50L Capacity
SS316 Vacuum Tray Drier – 12 trays
20L Rotovaps
3 fume hoods
2 Preparatory HPLCs (can be increased based on requirement)
Tray Lyophiliser (Virtis G35EL5) 35L capacity (2 numbers)
Class 100,000 clean room area

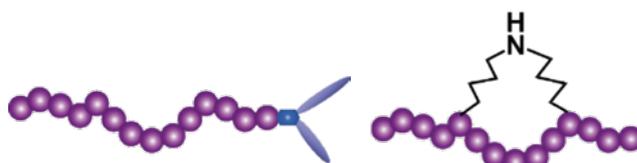


Neuland Laboratories R&D Building, Hyderabad, India

Jitsubo Co., Ltd.

Jitsubo Co. Ltd. (JITSUBO) is a Japanese pharmaceutical company that started in 2005 as a university-origin company from Tokyo University of Agriculture and Technology. Jitsubo focuses on research and development of peptide APIs. Jitsubo owns two original key technologies: **Molecular Hiving™** for production of high quality and cost-competitive peptide APIs, and **Peptune™** for novel peptide modification in drug discovery stages.

Molecular Hiving is a technology to manufacture peptides with innovative TAG-assisted liquid phase peptide synthesis (LPPS). **Peptune** is a novel peptide modification technology and can design much more stable and efficacy therapeutic peptides rather than those of traditional peptides. Jitsubo can provide to the global market not only research samples, IMPs, NCEs and generic peptide APIs with short lead time but also proposals of novel peptides for the drug discovery.



Molecular Hiving™
TAG-assisted LPPS

Peptune™ For detail:
www.jitsubo.com/en



Jitsubo's Life Science Research Center, Yokohama, Japan

CHARACTERISTICS

Molecular Hiving uses TAG, hydrophobic benzyl alcohol or benzyl amine derivatives at C-terminus, instead of resins in Solid Phase Peptide Synthesis (SPPS) method. The reactions of coupling to form peptides and de-protection of N-Fmoc or Boc in slightly hydrophilic solvent, e.g. THF, can be performed in homogeneous solution like in LPPS method. Precipitation and isolation of desired tagged-peptide can be performed easily by adding hydrophilic solvent, e.g. acetonitrile, to the reaction mixture. **Molecular Hiving** has advantages of both LPPS and SPPS.

ADVANTAGES

Molecular Hiving has advantages of both LPPS & SPPS.

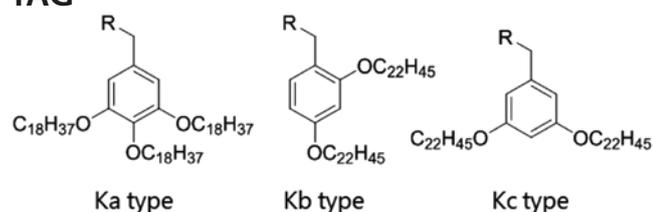
CRITERIOR	LPPS	SPPS	MOLECULAR HIVING™
1. Quality	✓		✓
2. Low-cost operation	✓		✓
3. In-process control	✓		✓
4. Short lead time		✓	✓
5. Easy separation		✓	✓
6. Long sequence		✓	✓

REACTION AND PRECIPITATION

All materials homogeneously solve in the reaction solution and only tagged-peptide can be isolated by precipitation.

REACTION mixture		In Slightly Hydrophilic Solvent, e.g. THF, DMF, including coupling reagents, by-product, and excess starting materials, etc.
PRECIPITATION		Appears after addition of Hydrophilic Solvent, e.g. acetonitrile,

TAG



R=OH For carboxylate at C-terminus of Peptide

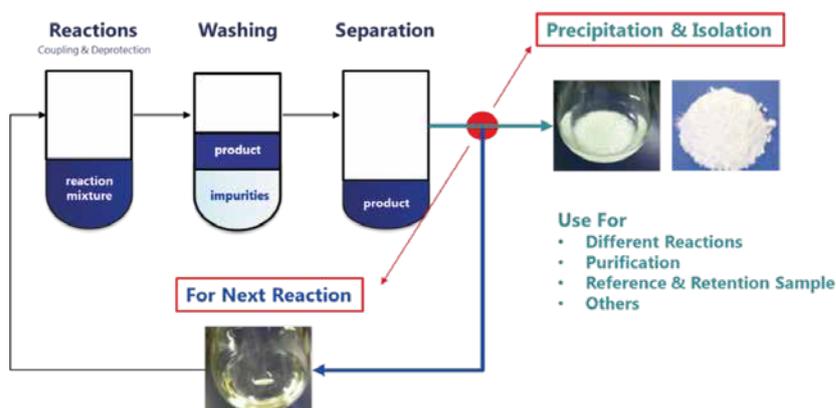
R=NH₂ For amide at C-terminus of Peptide

REFERENCES

- IK. Chiba, et. al., *J. Org. Chem.*, 2013, **78**, 320-327
- IWO2007/034812
- IWO2007/122847

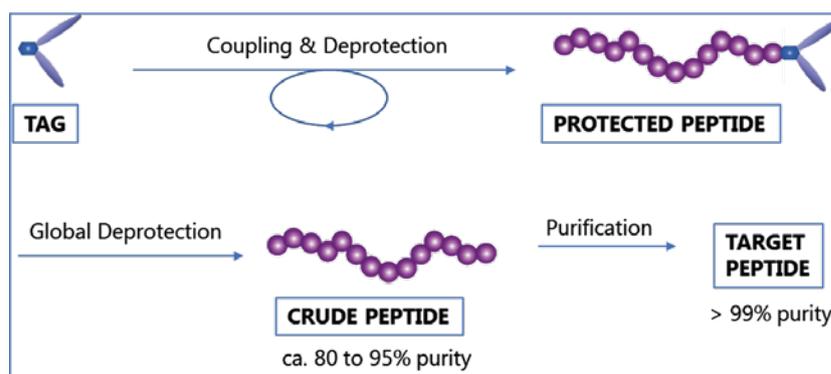
CAFETERIA-STYLE MOLECULAR HIVING

After removing the protected amino acid and other hydrophilic by-products in aqueous layer by separation, it is possible to choose whether to obtain the powder of the product or to go to the next amino acid elongation step. The organic layer includes hydrophobic impurities, e.g. dibenzofulvene derivatives when using N-Fmoc protected amino acids, however, they are inactive for the next reactions and possible to perform one-pot reactions until required numbers of amino acid.



GENERAL SYNTHETIC ROUTE USING ONE-POT METHOD

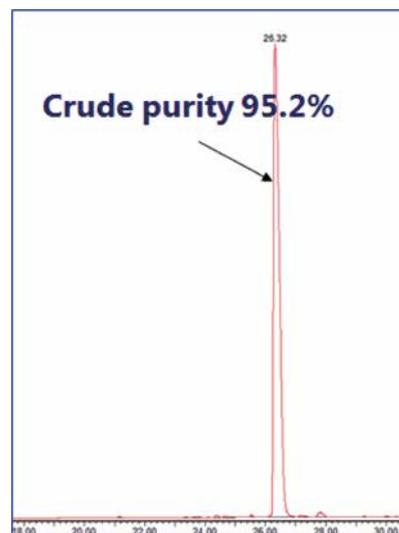
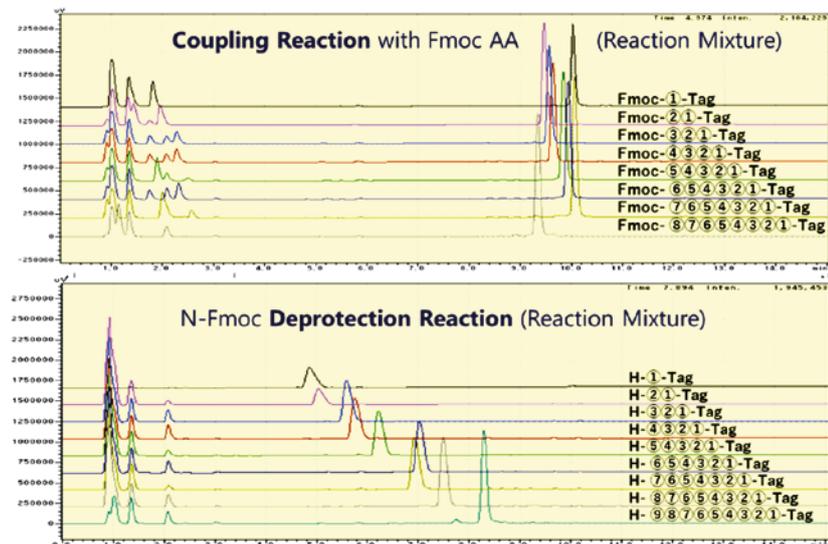
TAG is coupled with the first amino acid at C-terminus, which is usually N-Fmoc or Boc protected, and then 2nd to final amino acid at N-terminus can be elongated continuously. Numbers of amino acids that can be applied in one-pot method depend on sequence. Various coupling reagents can be used in **Molecular Hiving**. Reaction time is very short and operation per a step completes within 1 to 2h, therefore, 3 to 5mer/day elongation is possible by using the 'One-Pot Method'.



CASE STUDY

According to the One-Pot Method described previously, crude of PEPTIDE A (Linear, 9mer) can be obtained within 3 to 4 days with 95% of purity. Reactions can be well monitored by HPLC to control reaction-based impurities. After one purification step, 99.5% purity of target peptide is obtained.

IN-PROCESS CONTROL USING HPLC



Reversed-Phase Liquid Chromatography Using Surrogate (Additional) Stationary Phases (NeuCoat™)



CHARACTERISTICS

Neuland's patented surrogate stationary phase reversed-phase high performance liquid chromatography (SSP-RP-HPLC) utilize a hydrophobic quaternary ammonium salt (*NeuCoat™*) bound strongly to C-18 / C-8 reversed phase columns act as an additional stationary phase or surrogate stationary phase. Using Neuland's technology higher sample loading during preparative SSP-RP-HPLC (7 to 10X with respect to conventional preparative RP-HPLC) is achieved due to: (1) increase in the selectivity parameter, and (2) the increased stationary surface area (resultant as a lattice of hydrophobic quaternary ammonium salts adsorbed on the reversed phase column) that is available for interactions with the analytes (please see Table 1).

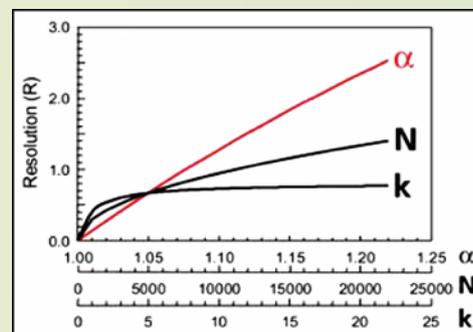


TABLE 1: SSP-Prep-RP-HPLC loading capacity is about 7-fold to 12-fold greater than conventional Prep-RP-HPLC (TBAHS is tetra-n-butylammonium hydrogen sulphate)

Purification of Leuprolide: Comparison of the Surrogate Stationary Phase aided Prep-RP-HPLC with the Standard Prep-RP-HPLC									
Entry #	Prep-RP-HPLC Method	Column Dimensions (ID X L)	Total Column Volume	Input: Crude API	Output: Pure API	% Yield	% Purity (USP HPLC Method)	mgs of Leuprolide (output)/ mL of Total Column Volume	Relative Loading Capacity
#1	Standard Prep-RP-HPLC	YMC-ODS-AQ (50 mm X 250 mm, C18, 10 μ, 120 Å pore diameter, 14 % C)	491 mL	4.0 g	1.20 g	30.0%	99.86%	2.4	1
#2	TBAHS-SSP-Prep-RP-HPLC	Waters Symmetry (19 mm X 50 mm, C8, 5 μ, 100 Å pore diameter, 11.7% C)	14.2 mL	1.4 g	0.42 g	30.0%	99.79%	29.6	12.1
#3	TBAHS-SSP-Prep-RP-HPLC	Discovery Bio Wide Pore (10 mm X 250 mm, C18, 5 μ, 300 Å pore diameter, 9.2% C)	19.6 mL	1.2 g	0.32 g	26.7%	99.73%	16.3	6.7

SSP-RP-HPLC is different from the traditional ion-pair chromatography (IPC): IPC involves pairing of the polar analyte with a hydrophobic cation or anion to increase its binding to the reversed phase C-18/ C-8 column. In the SSP-RP-HPLC the interactions are between the analyte and the SSP. The high impact of the SSP/ ASP on the overall resolution (please see Table 2) suggest a minor contribution from the C-18/ C-8 stationary phase which is serving as a scaffold for SSP. Also, the effect of the counter anion is minimal on the separation or the preparative output.

How tightly the SSPs are bound to the C-18/ C-8 reversed phase depends on the concentration of the organic eluent and the length of the alkyl chains comprising the SSP/ ASP. Weakly binding SSPs such as tetrabutylammonium salts need replenishing as the concentration of the organic mobile phase, example Acetonitrile, is increased because of the leaching of SSP from the reversed phase column.

Strongly binding SSPs such as **NeuCoat** do not need replenishing at acetonitrile concentrations below 80%. These SSPs bind very tightly to C-18/ C-8 alkyl chains of the reversed phase and to the residual silanols. Indeed with strongly bound SSPs traditional organic buffers such as Acetonitrile-Water-TFA- or aqueous acetonitrile containing triethylammonium phosphate, ammonium acetate- Aqueous Acetonitrile/ Methanol buffers are used.

The NeuCoat-SSP positively impacts the selectivity parameter and the overall resolution (please see Table 2). In most of the analytical RP-HPLC columns that were studied there was a significant improvement in the resolution "R".

TABLE 2: Coating Analytical Columns with NeuCoat improves the resolution "R" of O-Acyl Octreotide from Octreotide

Beneficial Effect of NeuCoat on the Resolution of Octreotide (Crude Sample) from a close eluting early peak with various columns											
Objective: Reduce "K" (shorter cycle time); Reduce "TF" (Narrower peaks); Increase "N" (Plate count/ efficiency); Increase "R" (Resolution); & Increase "α" (Selectivity)											
Column	"K": Capacity Factor (Average of 3 Runs)		"N" : USP Plates (Average of 3 Runs)		"R": USP Resolution (Average of 3 Runs)		"α": Selectivity (Average of 3 Runs)		"TF": USP Tailing Factor (Average of 3 Runs)		Comments
	Before NeuCoat	After NeuCoat	Before NeuCoat	After NeuCoat	Before NeuCoat	After NeuCoat	Before NeuCoat	After NeuCoat	Before NeuCoat	After NeuCoat	
YMC ODS-A	11.67	11.30	27575	31014	3.72	4.22	1.09	1.10	1.41	1.39	Reduction in K & TF. Increase in N, R, & α
Targa C8	12.05	11.05	16932	16433	1.67	2.18	1.04	1.06	1.76	1.68	Reduction in K & TF. Increase in R, & α
Vydac ODS TP21854	12.82	11.27	21790	16755	1.77	2.17	1.05	1.07	1.33	1.03	Reduction in K & TF. Increase in R, & α
Nucleosil 100-5-C18	14.04	11.59	28884	29005	2.29	4.02	1.05	1.10	1.68	1.73	Reduction in K. Increase in R, α, & TF
Inertsil ODS-2	11.26	10.22	18185	15299	3.39	3.31	1.12	1.14	1.17	1.38	Reduction in K, N, & R. Increase in α, & TF
Zobax SB-Phenyl	13.48	11.72	27731	27254	2.70	3.59	1.06	1.09	1.55	1.39	Reduction in K & TF. Increase in R, & α

The NeuCoat-SSP-RP-HPLC exceeds the sample loading capacity of even normal phase silica chromatography where the entire stationary phase is available for interaction with the sample being purified. The increased surface area available as a consequence of multiple layers of SSP that are available for interactions with the sample are partly responsible for the increased purification output. We have used this technique in our manufacture of 32 Kgs of a decapeptide, and for the purification of several other NCEs.



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