innovations 1 Vol. 12

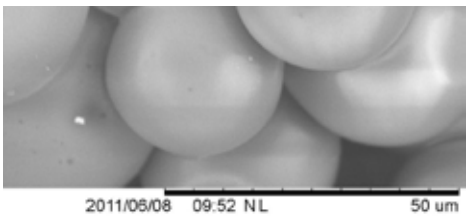
Novabiochem®
NEW • Spheritide resins

- Hydrophilic
- High-load
- Biodegradable
- Produced from sustainable materials
- Swell in wide range of solvents

NEW • High-load polar supports for SPPS

SpheriTide® resin is a novel hydrophilic, high-load support for both research and large-scale production of peptides that consists of poly-ε-lysine cross-linked with sebacic acid. In contrast to other supports that are manufactured from products derived from fossil fuels, both these starting materials are obtained from renewable biological sources. Poly-ε-lysine is a naturally occurring short-chain polyamide consisting of 25-35 lysine residues linked through their α-carboxyl and ε-amino groups that is produced by bacterial fermentation for use as a food preservative, whereas sebacic acid is made from castor oil for use in the textile industry. SpheriTide® is thus one of the first sustainable supports especially designed for peptide synthesis - it is also biodegradable as the polyamide backbone is susceptible to proteolysis.

a)



b)

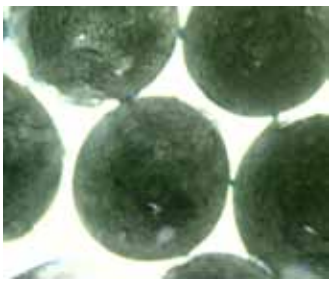
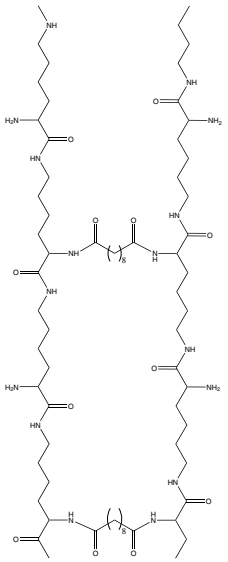


Fig. 1: a) Electron micrograph and b) light micrograph of SpheriTide® resin.

Fig. 2: Structure of SpheriTide® resin.



Properties of SpheriTide® resin

Features

- Loading of base polymer is approximately 3 mmol/g
- Loading of HMPA SpheriTide® resin is typical 1.8 - 2.2 mmol/g; Rink Amide SpheriTide® 1.0 - 1.3 mmol/g
- Low bed-volume to substitution ratio, means less solvent waste and lower excesses of reagents
- Ideal for research and large scale batch synthesis
- Compatible with a wide range of polar and nonpolar solvents, including MeOH, water, DCM, DMF
- Biodegradable - polymer is completely degraded by proteolysis
- Starting materials for resin derived from renewable resources

High-subsitution

Because SpheriTide® resin is essentially just a polymer of lysine, very high levels of functionality can be obtained without compromising its swelling and usability in peptide synthesis. Typically, the unloaded polymer has 3 mmol/g of reactive amino groups, which is approximately 2 times that of hydrophobic polystyrene resins and 3-10 times that of other comparable hydrophilic supports such as NovaPEG, PEGA and NovaSyn® TG. Due to addition of the mass of linker, loadings of HMPA SpheriTide® resin and Rink Amide SpheriTide® resin drop to 1.8 - 2.2 mmol/g and 1.0 - 1.3 mmol/g, respectively, which is still significantly higher than Wang and Rink Amide AM resins. This high level of substitution means bed-volumes can be kept to a minimum (Figure 1). This results in lower solvent consumption and allows the use of lower excesses of activated amino acids since more concentrated solutions can be employed.

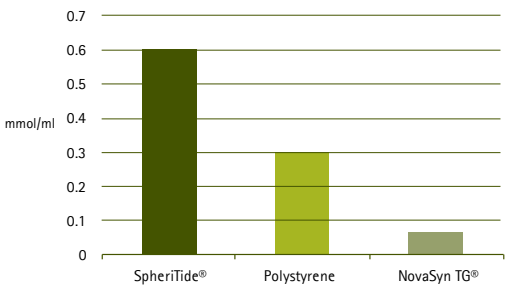


Fig. 3: Loading per ml in DMF for SpheriTide®, polystyrene and NovaSyn® TG resins.

Unique structural architecture

The uniform spacing of functional groups in SpheriTide® resin is unique and not chemically feasible in traditional polymers formed by free-radical polymerization. In resins made by radical polymerization and post-synthesis functionalization, the cross-linking and functionalization is not uniform throughout the polymer but tends to cluster. This leads to batch-to-batch irreproducibilty due to inconsistent levels of cross-linking, and introduces pockets of hindrance which ultimately leads to problems with formation of deletion and truncation sequences.

Swelling Properties

The polar backbone of the polymer allows the resin to swell in a wide range of solvents, including water, DMF, DCM and MeOH (Fig 4). However, it is important to note that because of the exceptional high loading of the polymer, the swelling properties of derivatized SpheriTide® resins will be largely determined by the nature of the attached peptide or linker.

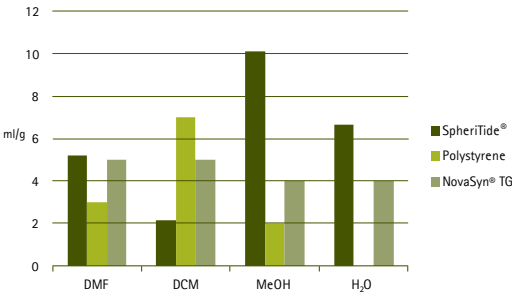


Fig. 4: Swelling volume of SpheriTide®, polystyrene and NovaSyn® TG resins in various solvents.

Example syntheses

Synthesis of ACP

Acyl carrier protein (65-74) is a classic example of a difficult sequence and is the standard test sequence to evaluate the utility of new resins for solid phase peptide synthesis. To compare SpheriTide® against polystyrene and polydimethylacrylamide (PDMA) resins, the manual synthesis of ACP(65-74) was undertaken using only 2-fold excesses of Fmoc-amino acids activated with TBTU with one hour coupling times on HMPA SpheriTide® (2.1 mmol/g), HMPA AM polystyrene (0.94 mmol/g) and HMPA-ethylenediamine-PDMA (0.84 mmol/g). Cleavage from the resin and side-chain deprotection of the assembled peptides were achieved by treatment with TFA/EDT (95:5).

Figures 5-7 show the HPLC profiles obtained for the crude products obtained from the three syntheses. The cleanest result was obtained using SpheriTide® resin, despite it having a substitution of almost twice that of the other resins tested.

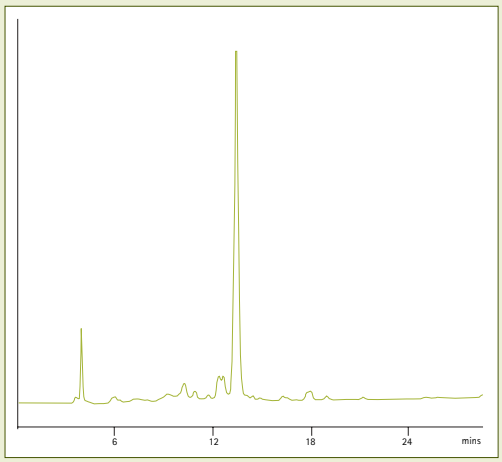


Fig. 5: HPLC profile of crude ACP(65-74) prepared with AM polysyrene resin.

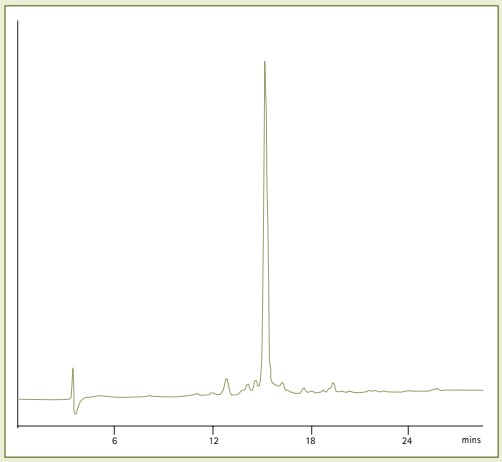


Fig. 6: HPLC profile of crude ACP(65-74) prepared with PDMA resin.

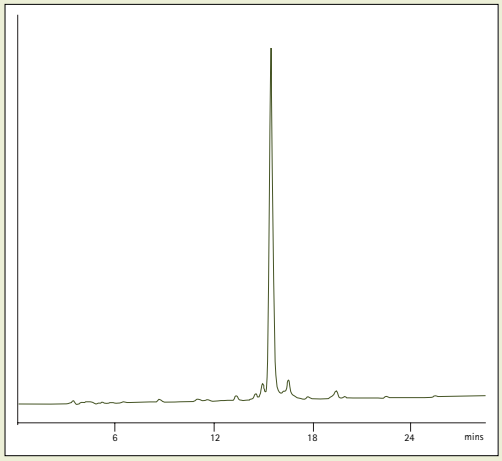


Fig. 7: HPLC profile of crude ACP(65-74) prepared with SpheriTide® resin.

Large scale synthesis of GLP analog

The 21 mmol scale synthesis of a 33 residue proprietary GLP analog was undertaken on AM polystyrene (1.07 mmol/g, 19.5 g) and SpheriTide® resin (3.0 mmol/g, 7g) functionalized with base-labile hydroxymethylbenzoic acid (HMBA) linker. Attachment of the C-terminal residue was effected using the symmetric anhydride in the presence of DMAP. Coupling reactions were conducted for one hour using only 2 equivalents of Fmoc-amino acid activated with DIPCDI/HOBt activation. Following coupling, unreacted amino groups were capped by treatment with Ac_2O/DMF. Prior to cleavage of the peptides from the resin, all side-chain protecting groups were removed with TFA/EDT. Release of the product peptide from SpheriTide® resin was achieved by saponification with 0.5 M NaOH aq. followed by neutralization with AcOH, to afford, after desalting, 48 g (98%) of the desired peptide (Figure 8). In the case of AM polystyrene, 0.5 M in methanol/water (1:1) was used for the saponification, owing to the poor swelling of the polystyrene resin in water, to provide only 31 g (62%) of the product (Figure 9).

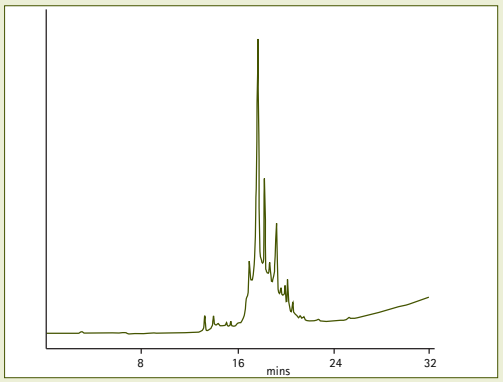


Fig. 8: HPLC profile of crude GLP analog prepared with polystyrene resin.

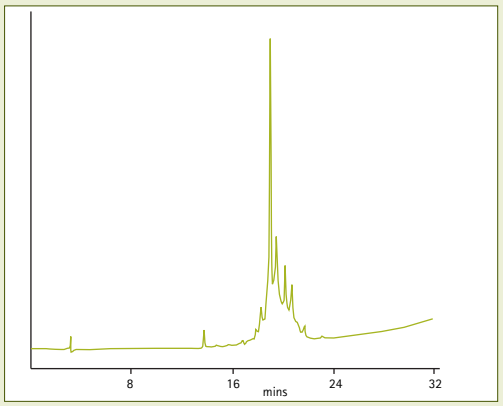


Fig. 9: HPLC profile of crude GLP analog prepared with SpheriTide® resin.

Conclusion

In this study, SpheriTide® resin proved superior to conventional polystyrene resin, affording the target peptide in significantly better yield and purity. Therefore, the high substitution, low-bed volume, biodegradability, and sustainability of SpheriTide® resin would appear to make it an excellent choice for the large scale solid phase synthesis of peptides.

Ordering Information

Cat.No.	Product	Contents	Price EUR
	NEW High-load polar supports for SPPS		
855150	HMPA SpheriTide resin	1g	54.00
		5 g	216.00
		25 g	864.00
855149	Rink Amide SpheriTide resin	1 g	70.00
		5 g	275.00
		25 g	995.00

For more information please contact our local offices:

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