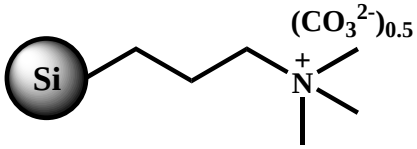
Use of Silia*Bond*® Carbonate

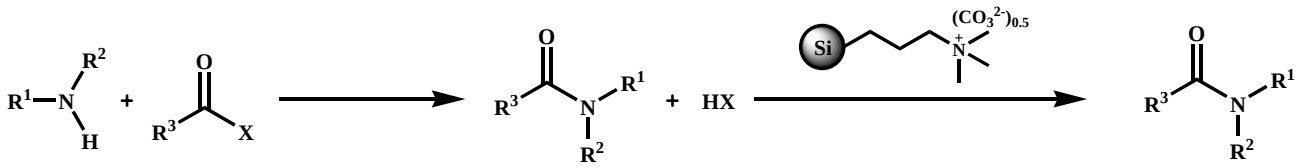


Silia*Bond*® Carbonate (or ***Si*-CO_3**) is the silica bound equivalent of tetramethylammonium carbonate. It can be used as a general base to quench a reaction, to free base amine hydrochlorides and to scavenge acids and acidic phenols, including HOBt, which is widely used in amide coupling reactions. **Silia*Bond*® Carbonate** is also very efficient at scavenging boronic acids: 99% of removal of phenylboronic acid with 1 equivalent in DCM at RT after 1 h.

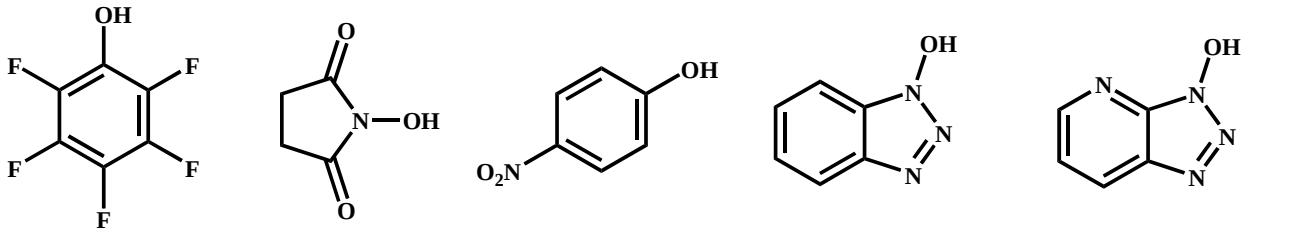
Amide Coupling

The use of functionalized silica allows to greatly simplify not only organic synthesis but also purification. In some cases, it permits to reduce the work up to a simple filtration and evaporation of the solvent. As we are developing more products and applications for amide synthesis, it became clear that **Silia*Bond*® Carbonate** could play a key role in further simplifying the work up in amide coupling reactions.

This application notes present different examples and results on the use of **Silia*Bond*® Carbonate** in the synthesis of amides using other **Silia*Bond*®** reagents. We investigated its efficiency as a scavenger of various coupling catalysts namely 1-hydroxybenzotriazole (HOBt), pentafluorophenol, 4-nitrophenol, 1-hydroxy-7-azabenzotriazole (HOAt), and *N*-hydroxysuccinimide (HOSu) and compared it with 3 suppliers of polymer-supported carbonate. We also looked at the yields and purities of different amide syntheses using **Silia*Bond*® Carbodiimide** and **Silia*Bond*® Carbonate**. In these reactions, the only work up consisted in a filtration and evaporation of the solvent. Lastly, we looked at the ability of **Silia*Bond*® Carbonate** to neutralize amine salts of various compositions.



where HX =



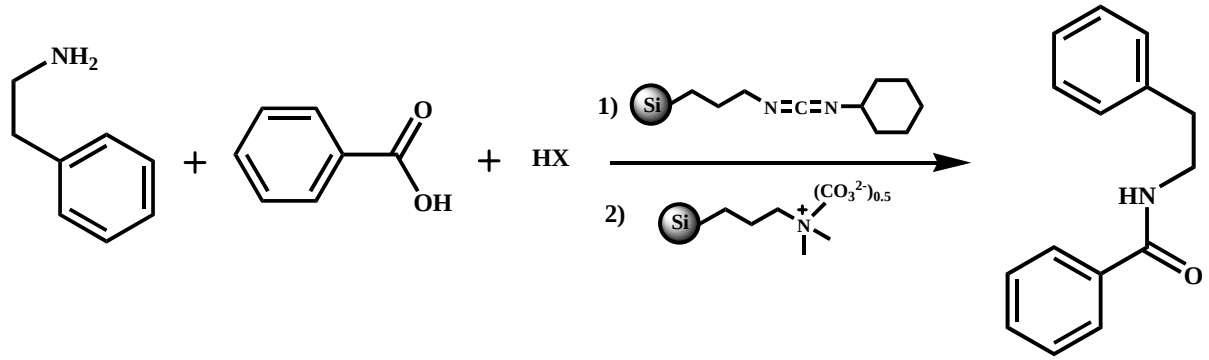
Pentafluorophenol **N-Hydroxysuccinimide** **p-Nitrophenol** **Hydroxybenzotriazole** **Hydroxy-7-azabenzotriazole**

	Residual concentration (ppm)								
	Silia*Bond*® Carbonate			Polymer 1		Polymer 2		Polymer 3	
	Bulk		Cartridges	Bulk		Bulk		Bulk	
HX	5 min.	60 min.		5 min.	60 min.	5 min.	60 min.	5 min.	60 min.
Pentafluorophenol[a]	2	2	3	8	5	15	6	1358	190
Hydroxysuccinimide	7	<5	<5	59	36	60	58	667	35
4-nitrophenol	6	4	<1	11	5	23	12	982	40
Hydroxybenzotriazole[b]	12	4	3	32	8	74	14	101	100
1-Hydroxy-7-azabenzotriazole[b]	3	3	3	28	4	70	8	3047	592

Initial concentration: 5,000 ppm – 3 eq. of **Silia*Bond*® Carbonate**. Analyzed by UV. [a]: analyzed by GC-MS; [b]: in THF

In the same conditions, silica always comes out as the most efficient scavenger. It is particularly efficient in cartridges.

The next step was to look at a synthesis of an amide including the scavenging of different coupling catalysts.



Catalyst (HX)	Yield (%)	Purity (%)
No catalyst[a]	35.4	95.1
Hydroxysuccinimide[a]	67.2	98.0
Hydroxybenzotriazole[b]	98.9	97.7
1-Hydroxy-7-azabenzotriazole[b]	100.0	99.2

1.0 eq. Amine, 1.5 eq. Acid, 1.7 eq. Catalyst, 2.0 eq. **Silia*Bond*® Carbodiimide**, 7.0 eq. **Silia*Bond*® Carbonate**. Yields refer to the mass of isolated product. Purity determined by GC-FID, [a]: in DCM, [b]: in THF

Free basing

Trifluoroacetic acid (TFA) is certainly the most commonly used ion-pairing agent for the separation of peptides in reversed-phase ion-pairing chromatography. This popularity is explained by the fact that:

1) it is volatile and easily removed;
2) it has low absorption within detection wavelengths;
3) it has a proven history.

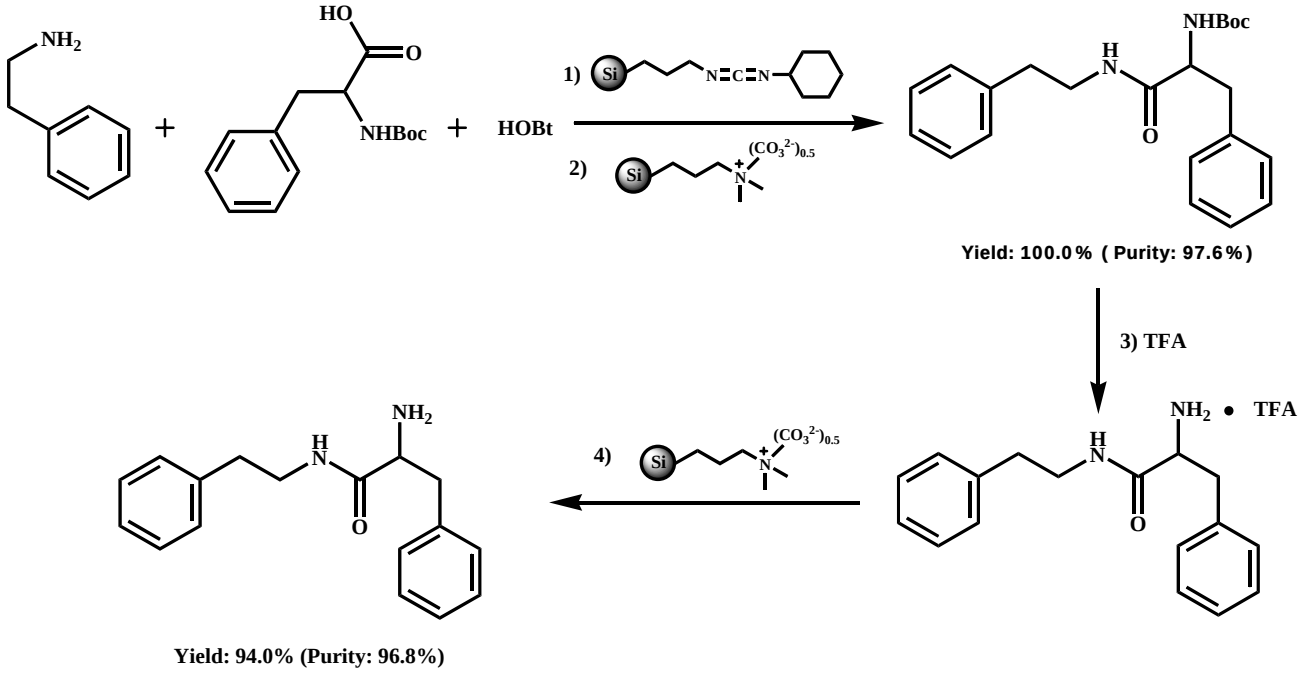
The role of TFA is to act as buffer, keeping the charge on the analyte and avoiding precipitation, to impart some hydrophobicity to the amino groups, and to neutralize cationic charges.

After HPLC separation, the peptide is isolated by evaporation of the solvent in its TFA salt form. Libraries of peptides stored this way exhibit reduced stability. It is hence necessary to use a method to free-base the peptides prior to their storage. **Silia*Bond*® Carbonate** is an efficient and convenient solution to this problem. We investigated the efficiency of **Silia*Bond*® Carbonate** to free base amines; we looked at different salts of ephedrine as presented in the following table:

Amine salt		Yield (%)	Purity (%)
Ephedrine	HCl	98.7	94.4
	TFA	100.0	98.9
	AcOH	100.0	99.2

In cartridges, MeOH, 4 eq. of **Silia*Prep*™ Carbonate**, eluted by gravity. Yields refer to the mass of isolated products; Purity determined by GC-FID.

It was then necessary to verify the efficiency of amine free-basing in a context of amide synthesis. We designed a reaction that would be more representative of real synthesis conditions and were the different applications of **Silia*Bond*® Carbonate** would be illustrated. In this particular example, we used the **Silia*Bond*® Carbodiimide** as the coupling reagent, followed by **Silia*Bond*® Carbonate** to remove HOBt. TFA salt of the amide was formed by the removal of the Boc protecting group.



Conditions

In bulk, THF 1) 1.0 eq. Amine, 1.5 eq. Acid, 1.7 eq. HOBt (Hydroxybenzotriazole), 2.0 eq. **Silia*Bond*® Carbodiimide**, 16 hours, 2) 7.0 eq. **Silia*Bond*® Carbonate**, 30 min., 3) 10% TFA in CH_2Cl_2, 16 hours, 4) 4.0 eq. **Silia*Bond*® Carbonate**, in CH_2Cl_2, 60 min.

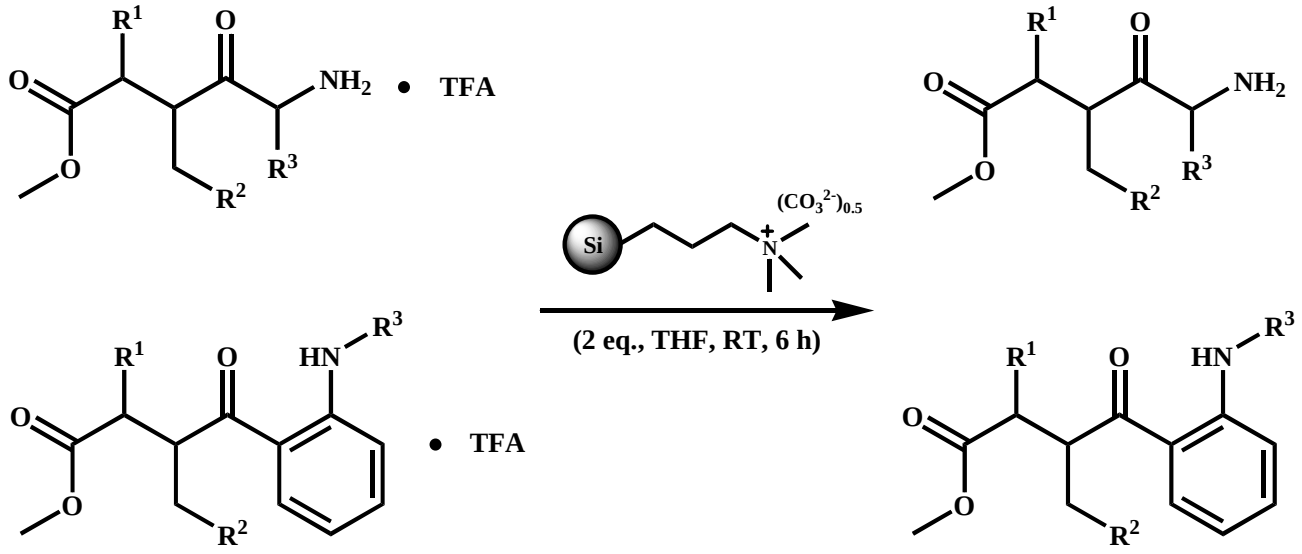
Yields refer to the mass of isolated product, purity determined by GC-FID

In an independent study, **Silia*Bond*® Carbonate** was tested side by side with MP – Carbonate for the scavenging of HOBt after an amide coupling reaction[1]. As shown in the following table, the silica-based scavenger has much faster kinetics, which allows it to completely sequester HOBt in only 5 minutes.

SPE media	Time (min)	HOBt removed[a] (%)
MP – Carbonate	120	77
MP – Carbonate	240	100
***Si* – CO_3**	5	100

[a]HOBt sequestration determined by LC/MS and verified by ^{1}H NMR

In another study, **Silia*Bond*® Carbonate** has been used to free base diversity libraries of 2,5-diketopiperazines and 1,4-benzodiazepine-2,5-diones[2]



[1] D. R. Sauer et al. *Org. Lett.* **2003**, 5(24), 4721-4724
[2] A. L. Kennedy et al. *Org. Lett.* **2002**, 4(7), 1167-1170