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Biopolymers **Hot Paper**

A New Way to Silicone-Based Peptide Polymers**

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Abstract: *We describe a new class of silicone-containing peptide polymers obtained by a straightforward polymerization in water using tailored chlorodimethylsilyl peptide blocks as monomeric units. This general strategy is applicable to any type of peptide sequences, yielding linear or branched polymer chains composed of well-defined peptide sequences.*

Hybrid copolymers composed of biomimetic and synthetic elements gather considerable attention as they open new and exciting perspectives both in the biomaterial and biomedical fields. Peptide-based hybrid copolymers are a class of biomimetic materials that significantly become important because of their specific properties such as biocompatibility, biodegradability, and bioactivity.[1–3] Recent studies focused on the preparation of well-defined homopolypeptides,[4] block copolypeptides[5–8,9] or more controlled architectures such as comb-like peptide polymers prepared by polymerization of original N-carboxyanhydride bearing a peptide sequence.[10] A second trend has emerged to yield hybrids of peptide and synthetic polymer, mainly with poly(ethylene glycol) and either (meth)acrylic chains[11] or with silica matrixes.[12–14] On the other hand, silicones, also referred as polysiloxanes, have a long track record as industrial biocompatible, bioinert, and biodurable materials.[15] Silicones have been widely used since the 60s in medicine as orthopedics, catheters, drains, shunts, extra corporeal equipments, implants, and as injectable fluids for soft-tissue augmentation.[16] However, these last properties are not sufficient for the more demanding tissue-engineering applications where additional bioactivity is required. This can be achieved by using organic–inorganic hybrids. For example, sugar–silicone or poly(L-lactic acid) (PLLA)–silicone hybrids have been reported for cell recognition and bone tissue regeneration.[17–19] The association of silicone and peptides has also been proposed for improved cell–material interactions purposes but it has been mostly limited to the functionalization of silicone surfaces with peptides.[20–22]

Herein we report on the synthesis of a new class of silicone biopolymers composed of tailored peptide sequences assembled through a one-step polymerization process initiated in water at neutral pH value. Noteworthy, this straightforward method does not require any post-grafting modification to obtain a functional polymer (Figure 1). The key point of the methodology was the synthesis of pseudopeptide monomers selectively functionalized by chlorodimethylsilyl (-SiMe$_2$Cl) moieties. Solution- and solid-phase synthesis strategies were successfully implemented for the preparation of such peptide building blocks. Interestingly, this methodology can be generalized to any peptide sequence.

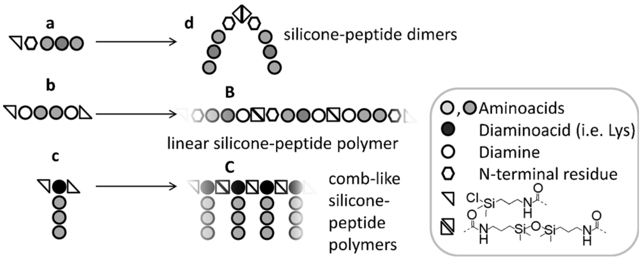

Figure 1. Silicone-containing peptide dimer **d** and polymers (**B** and **C**) obtained respectively from hybrid building blocks **a**, **b**, and **c**.

The chlorodimethylsilyl group (-SiMe$_2$Cl) was chosen for assembly of peptide-based polymers. Its reactivity has been extensively studied in the preparation of silicones.[23,24] Hydrolysis of chlorosilane (SiCl) to hydroxysilane (SiOH) groups followed by condensation yields siloxane bonds. The condensation occurs quickly in slightly basic media, at room temperature and in a wide range of solvents, advantageously in water. Interestingly, both reactions are completely chemoselective and take place in the presence of unprotected peptide side-chains (amines, carboxylic acids, alcohols, guanidine etc.). At last, a commercially available reagent (3-isocyanatopropyl chlorodimethylsilane) is used for the introduction of the chlorodimethylsilyl functionality onto peptides using their free amino groups.

For this study, three types of hybrid peptide building blocks were prepared (Scheme 1): 1) monofunctional hybrid peptides (type **a**) derivatized on their N-terminus and used for straightforward homodimerization (noted **d**); 2) bifunctional hybrid peptides (type **b**) functionalized both at the N- and C-terminus using a diamino derivative to yield linear polymers (noted **B**). Finally, Lys-bifunctional hybrid peptides (type **c**) gave a comb-like polymer (noted **C**), the peptide sequence of which is pendant on the main polymer chain (Figure 1). Five

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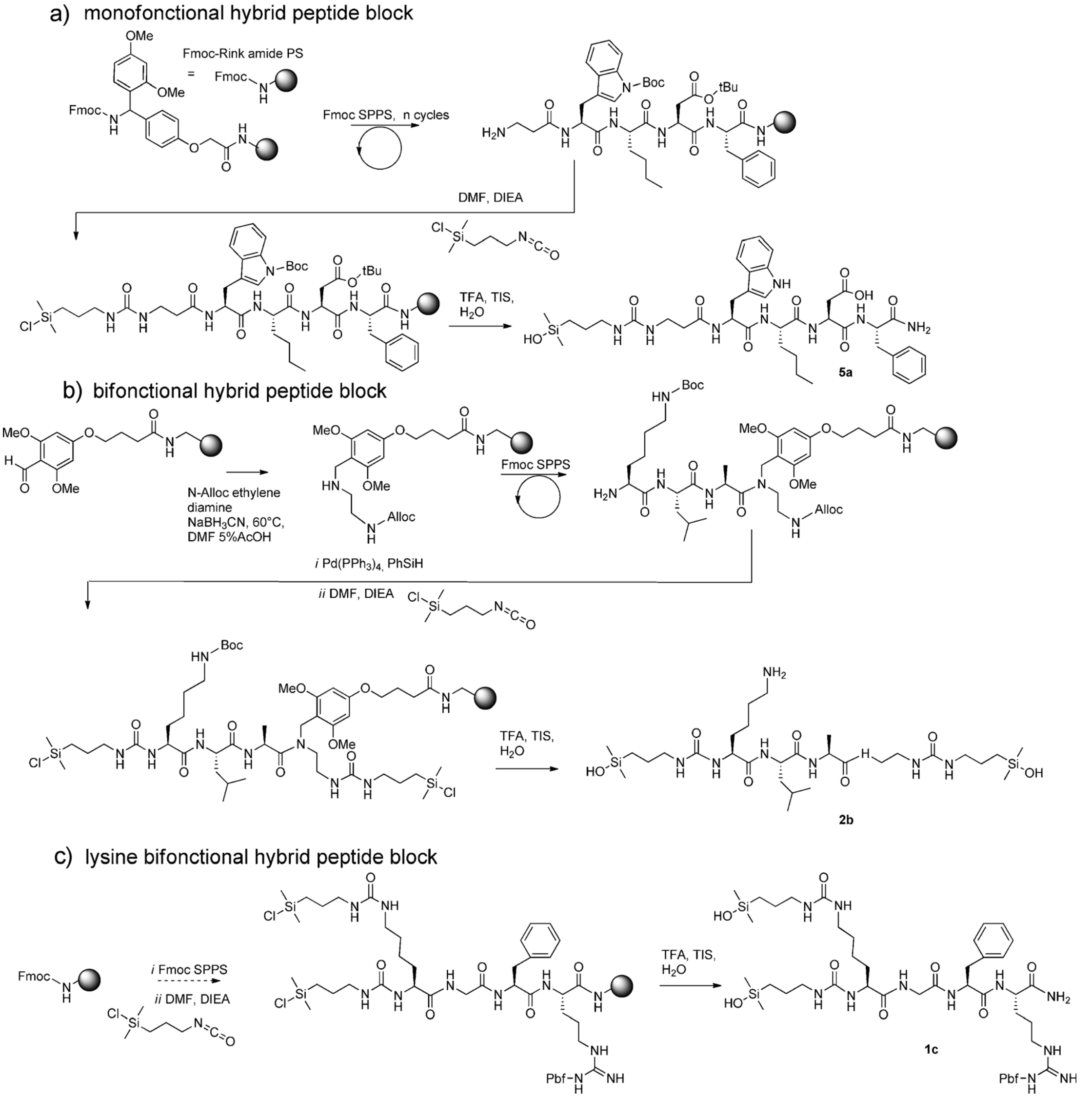

a) monofonctional hybrid peptide block

b) bifonctional hybrid peptide block

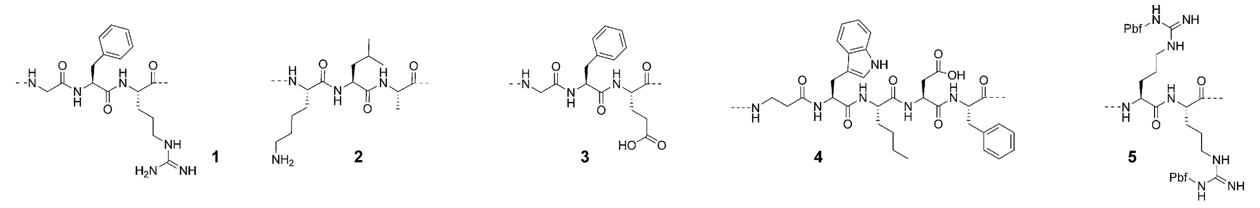
c) lysine bifonctional hybrid peptide block

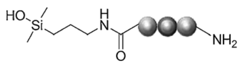
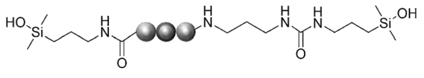
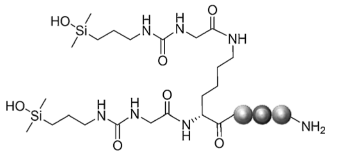
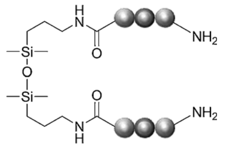
Scheme 1. Synthesis of dimethylhydroxysilane hybrid peptides. Way a) monofunctional hybrid peptide **a4** for dimerization; way b) linear bifunctional hybrid peptide **b2** for linear polymerization synthesis; way c) lysine bifunctional hybrid peptide **c1** for comb-like polymer synthesis. Fmoc = 9-fluorenylmethoxycarbonyl, SPPS = solid-phase peptide synthesis, and TIS = triisopropylsilane.

different sequences (**1** to **5**) were selected to cover most of the functional groups of unprotected amino acid side chains [i.e. aliphatic (Leu, Ala, Ile, Gly), aromatic (Trp, Phe), carboxylic acid (Asp and Glu), primary amine (Lys) and guaninidine (Arg)]. We also selected the protected sequence **5** bearing bulky and hydrophobic arylsulfonic derivative [Arg(Pbf)]. The combination of the five sequences with the three different types of blocks yields 15 hybrid dimethylhydroxysilane derivatives (Table 1).

Homodimerization of receptor ligands is a well-known strategy to improve binding affinity by increasing the local concentration of binders at the neighborhood of binding site. Enhancement of the biological effects was reported using dimeric analogues of numerous hormones (i.e. enkephalins,[25] neurokinins,[26] bradykinin,[27] serotonin[28]), of pharmaceutical drugs[29] and was also reported to inhibit protein–protein interaction.[30] The reported strategies mostly rely on the linking of a spacer arm on the solid support and the simultaneous synthesis of the two "arms" from the C to the N-terminus, on the coupling of protected peptides bearing a single free reactive function on the spacer or on chemoselective ligation.[31] In contrast, monofunctional hybrid peptide blocks **a** were dimerized chemoselectively in phosphate buffer (pH 7.4) at room temperature within two hours with good recovery yield (> 70%; **d1–5** in Table 1). Advantageously, no protection was required on the side chains, and the chlorodimethylsilane function could be introduced easily at the N-terminus (or at any point of the sequence through a lysine side chain). ^{29}Si NMR spectroscopy demonstrated that all silicon atoms are engaged in siloxane bonds. On the contrary, LC–MS analyses showed the presence of monomers and dimers because of hydrolysis of dimers in the acidic eluents. Siloxane bonds are stable in neutral conditions, enabling the dimerized compounds to be used in bioassays. As

Table 1: Hybrid peptides dimethylhydroxysilane blocks type **a**, **b** or **c** and dimer **d** obtained from block **a.** Hybrids blocks are noted *Xy*, with *X* being the type of block that is, **a**, **b** or **c**, and *y* being the sequence number, that is, **1** to **5**).



			1	**2**	**3**	**4**	**5**
a		Rt[a]	0′91″	0′87″	1′02″	1′42″	1′75″
		m/z[a]	537.5	489.3	510.0	809.5	994.6
		MW	536.7	488.3	509.6	809.0	993.3
b		Rt[a]	1′05″/1′35″	0′84″/1′11″	1′17″/1′55″	1′45″/1′75″	1′80″/2′16″
		m/z[a]	739.5/721.5	691.5/673.4	694.4/712.4	1011.0/993.8	1196.0/1177.1
		MW	739.0	691.0	711.9	1011.3	1195.6
c		Rt[a]	1′12″/1′46″	1′12″/1′46″	1′17″/1′57″	1′45″/1′79″	1′75″
		m/z[a]	824.6/806.5	776.5/758.5	797.5/779.5	1096.7/1078.8	1281.0
		MW	824.1	776.1	797.0	1096.4	1280.7
d		Rt[a]	1′23″	1′18″	1′43″	1′83″	2′43″
		m/z[a]	1055.7	959.7	1001.7	800.9	984.9/657.4
		MW	1055.4	959.4	1001.2	1599.9	1968.6

[a] Intermolecular condensation lead to dehydrated forms and can be observed during ESI$^+$ LC–MS analysis. Retention times (Rt) and *m/z* values of both species are presented: *m/z* $[M+H]^+$/*m/z* $[M-OH]^+$. MW = molecular weight.

a proof of concept, the binding affinity of dimer **d4** that displayed two copies of the cholecystokinin (CCK) binding minimal sequence Trp-Nle-Asp-Phe was determined on CCK_B receptors on Jurkat cell line.[32] With the C-terminus amide being particularly important for receptor binding, dimerization was achieved at the N-terminus. Displacement experiments of the reference radiolabelled ligand 3-[^{125}I]iodo-4-hydroxyphenyl-propionyl-CCK ($K_d = 0.5$ nM) were performed. Dimer **d4** has a K_i of 4.9 nM versus 18.9 nM for the monomeric sequence H-βAla-Trp-Nle-Asp-Phe-NH_2 and 0.26 nM for the CCK-8 reference ligand H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2[33] (Figure 2).

Hybrid blocks **b1–5** generated linear polymers **B1–5** through condensation between two SiOH groups. Hybrid blocks were dissolved in a 1/1000 v/v trifluoroacetic acid (TFA)/water solution (0.5 g mL^{-1}). Phosphate buffer (pH 7.4) was added dropwise under stirring to slowly increase the pH of the reaction mixture above 7. Condensation occurred during buffer addition and induced precipitation of the polymer. Comb polymers **C1–5** were obtained from hybrid blocks **c1–5** with the same procedure. Only comb polymer **C1** remained soluble upon buffer addition. Occurring in water, polymerization stops quickly when the polymer starts to precipitate. Thus the degree of polymerization is limited by the solubility of the growing polymer in buffer, thus depending on the nature of the peptide sequence. Longer chains could possibly be obtained using solvent mixtures such as dimethyl formamide (DMF)/water. In any case, the medium was freeze-dried and polymers were obtained as a powder or a sticky gel. The polymers were insoluble in most solvents (i.e. DCM = dichloromethane, MeOH, THF = tetrahydrofuran, EtOH, chloroform) while **B4**, **C4**, **B5**, and **C5** were soluble in dimethyl sulfoxide (DMSO) and DMF. Thermal stability of monomers and their corresponding polymers was studied by thermogravimetric analysis (TGA) from room temperature to 800 °C, under air with 5 °C min^{-1} heating rate. In all case, the inception of the thermal decomposition was detected from about 200 °C until complete oxidation into silica at about 800 °C (Supporting Information). From thermolysis curves, we could perceive, as expected, that polymers were more stable than their corresponding monomers, while no significant difference appeared between monomers and dimers. For the polymers, the general trend involved a two-step thermolysis. An initial 60–70 % weight loss was observed between 200 °C and 500 °C, followed by a 15–25 % weight loss between

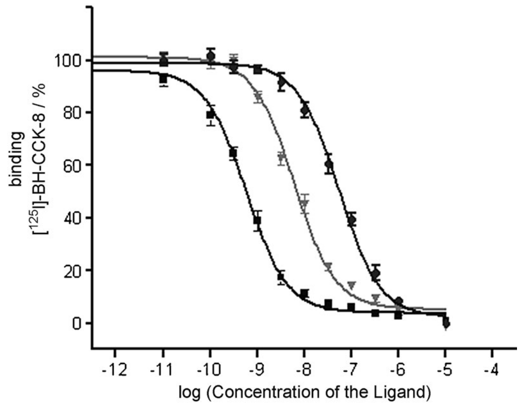


Figure 2. CCK_B Receptor binding of H-βAla-Trp-Nle-Asp-Phe-NH_2 (▼); **d4** (●) and H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (■).

500 and 800 °C. All compounds presented residual weight due to the inorganic matrices (SiO_2) roughly in agreement with the theoretical calculated values. Comb polymers **C1–5** had improved thermal stability compared to their linear counterparts **B1–5**. Again this might be attributed to improved interactions taking place between the pending peptides of polymers **C1–5** as a consequence of their higher spatial proximity in comb polymers compared to the linear ones.[34]

Glass-transition temperatures (T_g) were determined by differential scanning calorimetry (DSC) analyses, which were performed on monomers **a**, **b** and **c**, on dimers **d** and on polymers B and C from −60 °C to 150 °C (Table 2). All T_gs, when detectable, were in the range 25 to 50 °C, much higher than for classical silicones as an expected consequence of the hybrid peptide structure of the polymers. No clear influence of the polymer type was observed.

Table 2: Analyses of silicone-containing peptide polymers **B1–B5** and **C1–C5**.

polymer	T_g [°C][a]	M_n[b]	Đ[b]	DP_{SEC}[b]	DP_{NMR}[c]
B1	+30	insoluble[d]	insoluble	insoluble	insoluble
B2	+24; −41	insoluble	insoluble	insoluble	insoluble
B3	+25; −39	insoluble	insoluble	insoluble	insoluble
B4	no T_g	19100	3.9	19	30
B5	no T_g	23300	1.27	20	18
C1	+49	16900	1.24	21	28
C2	+26	insoluble	insoluble	insoluble	insoluble
C3	no T_g	insoluble	insoluble	insoluble	insoluble
C4	no T_g	7050	1.44	6	6
C5	+24	16700	1.07	13	16

[a] T_gs were determined by DSC with a temperature ranging from −60 to +150 °C. [b] M_n (g mol^{-1}), polydispersity index Đ and DP were estimated by SEC (in DMF) using PS as standard. [c] DPs were estimated by ^{29}Si NMR spectroscopy by comparison of the integrals of SiOSi (7.9 ppm) and SiOH (11.5 ppm) signals. [d] Not determined, insoluble polymer.

Finally, for **B2** and **B3** a second T_g was seen at −40 °C. As a consequence of the insolubility these polymers only hypotheses can be drawn, however this low T_g may be attributed to the presence of cyclic species containing only SiOSi bonds. Soluble polymers were analyzed by steric exclusion chromatography (SEC; Figure 3) and ^{29}Si NMR spectroscopy. SEC chromatograms clearly showed that type B linear polymers polymers had significantly higher molecular weight than their type C counterparts. As an example M_n(**B4**) is about 25000 and M_n(**C4**) is about 7600 g mol^{-1} (Table 2; M_n = number average molar mass). This corresponded to an average polymerization degree (DP) of about 19 versus about 6 for B4 and C4, respectively. In opposition to **B** linear polymers, the polymerization degree of type **C** comb polymers seemed strongly impacted by the peptide sequence probably because of steric hindrance of pendant chains upon polymerization. ^{29}Si NMR spectra (Supporting Information) of polymers **B4**, **B5**, **C1**, **C4**, and **C5**, which were soluble in DMSO were recorded. Integration of the signal at 7.9 ppm assigned to M^1 substructures, which corresponded to silicon atoms involved in SiOSi bonds, as well as integration of the signal at 11.5 ppm (M^0 substructures) related to silanol end groups (SiOH), were used to calculate the SiOSi/SiOH ratio, which corresponded to half of the polydispersity index estimated by NMR spectroscopy (DP_{NMR}). Ratios were in good agreement with SEC analyses. As example, for **B5** DP_{NMR} was 18 (19 as determined by SEC), whereas for **C5** DP_{NMR} was 16 (13 determined by SEC). For polymer **B4**, the resolution of the NMR spectra did not permit to detect and quantify precisely the SiOH peak, suggesting that the ratio was about 15/1 corresponding to DP > 30. However, for this sample the NMR integration had to be commented carefully as the presence of cyclic species, containing only SiOSi bonds could not be excluded.

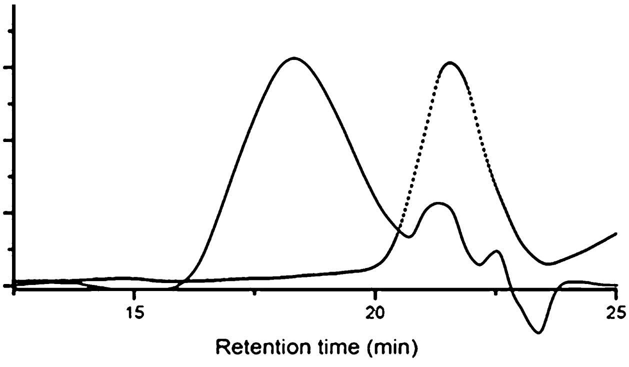


Figure 3. SEC chromatograms (DMF, LiBr 1 %) of polymer **C1** (plain line) and monomer **c1** (dotted line; UV detection at 254 nm).

For the first time, we propose a bottom-up approach leading to an oligomerization of peptide sequences by straightforward hydrolytic condensation process. Occurring in water, at room temperature and requiring neither catalyst nor chemical reagent, this type of polymerization could be scaled up easily. The diversity of structures and functions of peptides associated with the possibility to mix different hybrid blocks at different ratios, open the door toward multifunctional biopolymers that should find numerous applications, for example as systems for drug or gene vectorization and delivery, scaffolds for tissue engineering or antibacterial films.

Experimental Section

Hybrid peptide synthesis: All peptides were synthesized by the Fmoc/*t*Bu strategy using DMF as solvent.[30] After selective removal of protecting groups from amino group(s), peptides are derivatized with 3-isocyanatopropyl chlorodimethylsilane in the presence of *N*,*N*-diisopropylethylamine (DIEA) in DMF. Introduction of the dimethyl chlorosilane moiety could be achieved either in solution (Supporting Information) or on the peptide still anchored on the solid support. In this case, the excess of silylating agent was removed by filtration and washing steps. The choice of the solvent was guided by the good swelling of polystyrene (PS) resins and the good solubility of protected amino acids. Special attention was paid on the dryness of DMF used during isocyanate reaction since premature hydrolysis could decrease the reaction yield.

Monofunctional hybrid peptides **a1–4** were obtained after N-terminus silylation and cleavage from Rink amide resin (Scheme 1 A). The same approach was used for the synthesis of Lys-bifunctional hybrid peptides (**c1–3**, Scheme 1 C) for which an extra Lysine residue was added to the N-terminus of the sequence to be "comb"-polymerized, the two α and ε amino functions being simultaneously deprotected and silylated with ICPCDS on solid support. N- and C-ter bifunctional hybrid peptide blocks (**b1**–**5**) were either prepared on a 2-chlorochloro-trityl PS resin, cleaved and silylated in solution, or obtained by a solid support strategy using the backbone amide linker [5-(4-formyl-3,5-dimethoxyphenoxy)pentanoyl] resin (Scheme 1B). After final deprotection/cleavage, blocks obtained as hydroxydimethylsilyl species were analyzed by LC–MS and purified by RP HPLC. Hybrid blocks were soluble in water/acetonitrile (1/1 v/v) 1/1000 TFA. These acidic condition solution (pH 2.5) strongly prevents the oligomerization. Indeed, only very low amounts of intramolecular condensation or dimers (in the case of type **a** compounds) are detected (ca. 5%). However, even if they were easily separated by preparative HPLC at different retention times, the collected fractions always contained free (Si-OH) and condensed blocks (Si-O-Si) in equilibrium. This behavior had no significant impact on the further polymerization or dimerization process. After preparative HPLC, hybrid blocks were dehydrated by freeze-drying and can be stored at 4°C for months.

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