DEUSS: A Perdeuterated Poly(oxyethylene)-Based Resin for Improving HRMAS NMR Studies of Solid-Supported Molecules

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Abstract: A novel resin called DEUSS (per**deu**terated poly(oxyethylene)-based **s**olid **s**upport) has been prepared by anionic polymerization of deuterated [D_4]ethylene oxide, followed by cross-linking with deuterated epichlorohydrin. DEUSS can be suspended in a wide range of solvents including organic and aqueous solutions, in which it displays a high swelling capacity. As measured by proton HRMAS of the swollen polymer, the signal intensity of the oxyethylene protons is reduced by a factor of 110 relative to the corresponding nondeuterated poly(oxyethylene)poly(oxypropylene) (POEPOP) resin, thus facilitating detailed HRMAS NMR studies of covalently linked molecules. This ^{1}H NMR invisible matrix was used for the solid-phase synthesis of peptides, oligoureas, and a series of amides as well as their characterization by HRMAS NMR spectroscopy. On-bead NMR spectra of high quality and with resolution comparable to that of liquid samples were obtained and readily interpreted. The complete absence of the parasite resin signals will be of great advantage, for example, for the optimization of multistep solid-phase stereoselective reactions, and for the conformational study of resin-bound molecules in a large variety of solvents.

Keywords: combinatorial chemistry • NMR spectroscopy • oligoureas • peptides • poly(oxyethylene) • resins

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Introduction

Introduced by Merrifield in 1963, the polystyrene resin is certainly the most widely used polymer for solid-phase organic and peptide synthesis.[1] In the last decade, however, there has been a sustained research effort to develop new polymers with alternative physicochemical properties. For example, biocompatible solid supports with increased polarity were required for improving on-bead biological assays.[2] For this purpose, resins containing poly(ethylene glycol), such as Tentagel, PEGA, poly(oxyethylene)poly(oxypropylene) (POEPOP), and superpermeable organic combinatorial chemistry resin (SPOCC), have been conceived and prepared.[3] These polymeric materials also proved to be of great utility for the characterization of the molecules covalently linked to the insoluble matrix by using HRMAS NMR spectroscopy.[4] Some of them greatly improved the quality of HRMAS spectra allowing to reach a resolution very close to the NMR spectra obtained in solution.[5] However, the proton signals of the resin often cover part of the spectrum and overlap with the resonances of the bound molecule.[4a,c,5a] In this case, the assignment of the protons and the identification of the structure of the compound are not straightforward. In addition, the analysis of NOESY and ROESY proton HRMAS spectra is generally complicated

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by the appearance of correlation peaks between the resin and the molecule, due to spin diffusion phenomena and direct dipolar interactions involving the proton of the linked molecule close to the resin. Likewise, NMR spectra can be affected in experiments in which resin signals are saturated. Indeed, the saturation experiment of resin signals induces a loss of spin magnetization of the bound molecule close to the resin. This is due to the spin diffusion and renders the spectra nonquantitative. Moreover, signal saturation gives rise to different undesired artefacts in 2D NMR spectra. NMR spectra can also be perturbed when double saturation experiments for the suppression of the signals arising from the resin and the solvent are performed. For these reasons, the elimination of the matrix peaks from the HRMAS spectrum would be of great advantage.

About fifty years ago, deuterated solvents revolutionized the NMR technique, since they opened the possibility of acquiring spectra devoid of the contribution of the solvent protons. More recently, per-deuteration of proteins has considerably improved the NMR investigation of high molecular weight systems.[6] Bearing these ideas in mind, we have conceived and developed a novel solid support based on a cross-linked perdeuterated poly(ethylene glycol) (PEG) chain. We have studied the utility of this resin for the solid-phase synthesis of peptides, non-natural oligomers, and small organic molecules (e.g., amidated amino acids). In addition, we have demonstrated that this ^{1}H NMR invisible polymer greatly enhances the HRMAS NMR study of the resin-bound molecules.

Results and Discussion

Fully deuterated poly(oxyethylene) polymers were prepared from [D$_4$]ethylene oxide by using a slightly modified procedure to that reported by Eisenbach and co-workers.[7] We have synthesized two precursors with an average molecular weight of 1600 and 2000, respectively, which were characterized by GPC and ^{1}H NMR spectroscopy. These polymers were subsequently cross-linked upon derivatization with deuterated epichlorohydrin. Following the method of Meldal,[3d] we obtained the resin DEUSS (per**deu**terated poly(oxyethylene)-based **s**olid **s**upport) by bulk anionic reaction (Figure 1, inset). This resin has the same molecular structure as the cross-linked POEPOP resin.[3d] Resin micropellets were prepared by chopping the bulk polymer into small rectangular pieces, selected on size by sieving. In this study we have used DEUSS based on poly(ethylene glycol) 1600. The resin was characterized by proton and deuterium HRMAS spectra of the swelling polymer in chloroform and dimethylsulfoxide, and by MAS NMR spectroscopy of the dry polymer (Figure 1 and Supporting Information).

The residual proton signals are located at about 3.55 ppm. Preliminary studies have shown that DEUSS can be suspended in a wide range of solvents, including organic and aqueous solutions. We have noticed that this resin presents a swelling capacity higher than that of the cognate POEPOP (data not shown). The amount of the reacting groups (loading) on the resin was calculated by coupling an Fmoc-protected glycine, followed by the cleavage of Fmoc with piperidine and spectrophotometric determination of dibenzofulvene adduct absorbance at 300 nm. The loading was about 0.55 mmol g^{-1}. The hydroxy groups of DEUSS **1** were transformed into amines by using the Mitsunobu procedure already described for POEPOP-OH (Scheme 1).[4c,8]

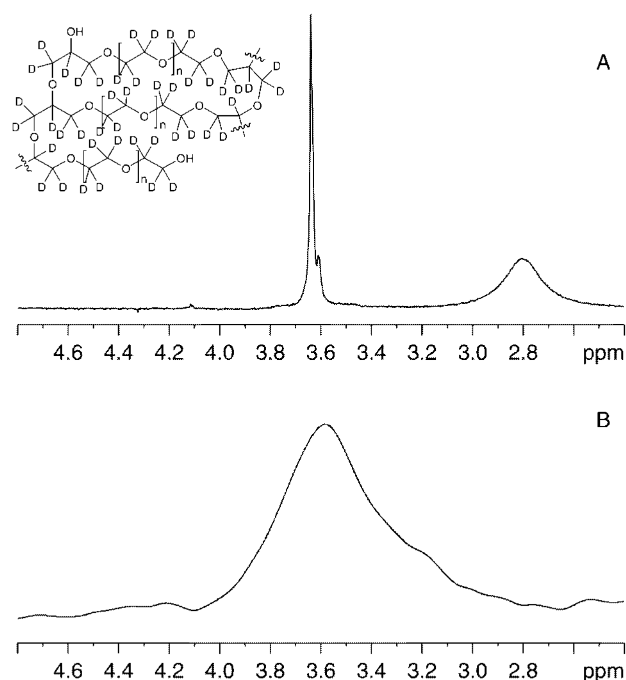
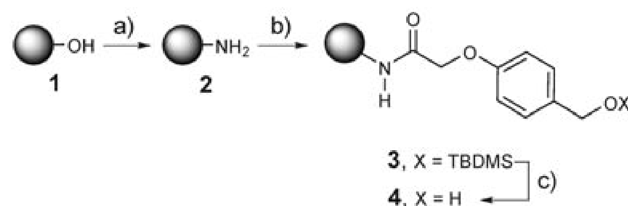


Figure 1. A) ^{1}H and B) ^{2}H HRMAS NMR spectra of DEUSS **1** swollen in CDCl$_3$, spun at 7 KHz. Inset: molecular structure of DEUSS **1**.



Scheme 1. Derivatization of DEUSS **1**: a) i) PPh$_3$/phthalimide/DIAD in CH$_2$Cl$_2$/THF, ii) N$_2$H$_4$ in NMP according to ref. [4c]; b) 4-(*tert*-Butyldimethylsilyloxymethyl)phenoxyacetic acid, BOP/HOBt/DIEA in DMF; c) 1 M TBAF in THF.

After this reaction, the NH$_2$ content was estimated to be 0.50 mmol per gram of resin. The amino functions of DEUSS **2** were easily derivatized with an acid-labile linker (Wang-like) by coupling 4-(*tert*-butyldimethylsilyloxymethyl)phenoxyacetic acid through activation with benzotriazole-1-yloxytris(dimethylamino)phosphoniumhexafluorophosphate (BOP)/1-hydroxybenzotriazole (HOBt)/diisopropylethylamine (DIEA) in dimethylformamide. Figure 2 shows the comparison between the HRMAS spectra of DEUSS **3** (Scheme 1) and the corresponding nondeuterated POEPOP swollen in CDCl$_3$. The greatest difference is certainly evidenced by the almost complete absence of the resin resonance at 3.55 ppm. The signal intensity of the oxyethylene

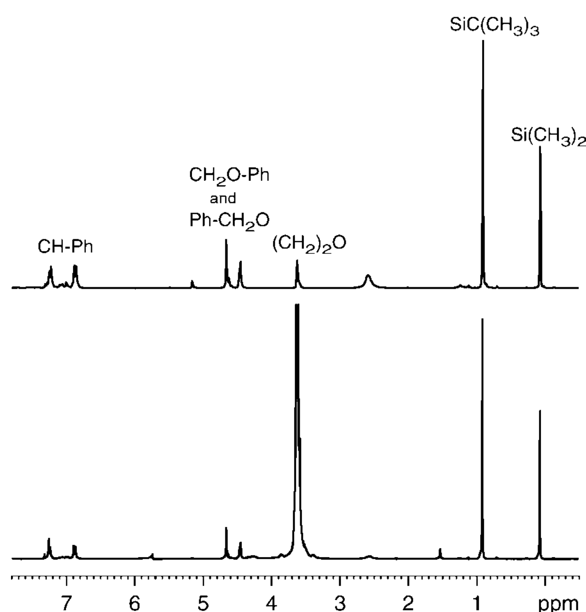
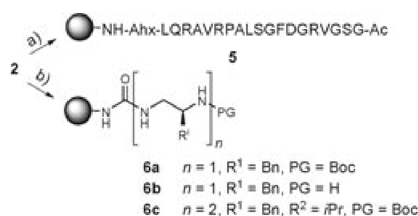


Figure 2. ^{1}H HRMAS NMR spectra of DEUSS **3** (top) and nondeuterated POEPOP (bottom) functionalized with the same linker, swollen in $CDCl_3$.

protons is reduced by a factor of 110 passing from POEPOP to DEUSS. This feature facilitates the identification and the assignment of the proton resonances of the covalently linked molecule. It is worth mentioning that the resolution of the HRMAS spectrum recorded on DEUSS is also improved relative to that recorded on the POEPOP resin (vide infra), and it is not necessary to use saturation experiments to eliminate the resin signals.

The *tert*-butyldimethylsilyl protecting groups of DEUSS **3** were subsequently removed by using a solution of TBAF in THF. We have observed that in the HRMAS spectrum of resin **4** the hydroxymethyl function was present as a tetrabutylammonium salt, despite extensive washing. Treatment with a 5% acetic acid solution in dichloromethane was required to liberate the OH group. Each transformation of the fully deuterated resin was also followed by ATR-FT-IR spectroscopy. The characteristic C–D stretching band of DEUSS appears at 2185 (broad) and 2081 cm^{-1} (sharp) (see the Supporting Information).

DEUSS **2** was used for the manual or automated synthesis of a series of peptides of different length including the 141–159 sequence from VP1 protein of foot-and-mouth disease virus (FMDV) (Scheme 2).[9] The solid-supported peptide **5**



Scheme 2. Synthesis of peptide– and oligourea–DEUSS conjugates: a) automated solid-phase synthesis of peptide according to ref. [4c]; b) step-by-step synthesis according to ref. [10].

was characterized by homo- and heteronuclear two-dimensional HRMAS NMR spectroscopy. In particular, Figure 3A displays the partial ^{1}H–^{13}C HSQC spectrum of FMDV peptide/DEUSS conjugate **5** swollen in deuterated dimethylformamide. The dispersion of the signals is identical to that of the same peptide bound to POEPOP.[4c] This suggests that the FMDV peptide probably adopts the same conformation on DEUSS and POEPOP.[4c]

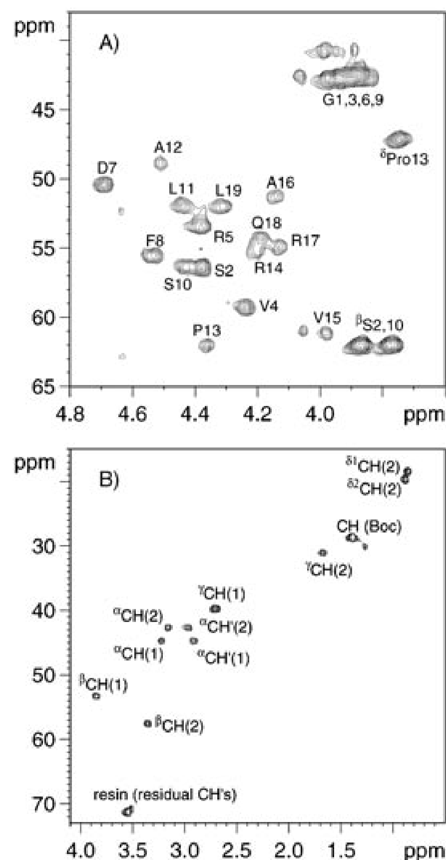
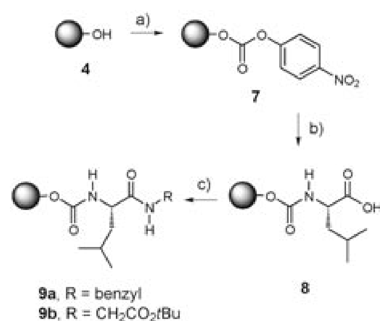


Figure 3. Partial ^{1}H–^{13}C HSQC of DEUSS-supported peptide **5** (A) and oligourea **6c** (B) swollen in [D$_7$]DMF and CD_3CN, respectively.

Further interest in DEUSS certainly stems from the possibility to rapidly analyze the folding propensity of non-natural oligomers (i.e., foldamers)[10] as a function of their sequence and chain length in various solvents. We have prepared a series of model mono- and diureas (**6**) on DEUSS **2** (Scheme 2).[11] Spin systems in **6c** were nicely resolved by using a combination of TOCSY and ^{1}H–^{13}C HSQC experiments in CD_3CN (Figure 3B). The chemical shift differences between diastereotopic H–C(α), an important parameter used to probe conformational homogeneity in helical N,N′-linked oligoureas, can be extracted directly from the ^{1}H–^{13}C HSQC spectra of the resin-bound oligoureas.[12]

Finally, we wanted to demonstrate the potential of the new perdeuterated resin in the field of organic and combinatorial synthesis of small molecules.[13] For this purpose, we have developed the multistep solid-phase synthesis of the secondary amides **9** (Scheme 3). DEUSS **4** was initially activated as a 4-nitrophenyl carbonate (**7**) and subsequently re-



Scheme 3. Synthesis of small organic molecules: a) 4-nitrophenylchloroformate/NMM in CH_2Cl_2; b) i) H-Leu-OH, P_2-Et in THF, ii) washing with 10% AcOH in CH_2Cl_2; c) BOP/HOBt/DIEA, R-NH_2 in DMF.

acted with leucine in the presence of phosphazene base P_2–Et to give **8**. As previously described by Palomo et al.,[14] phosphazene bases (P_1–*t*Bu, P_2–Et, and BEMP) efficiently promote solubilization of zwitterionic amino acids in organic solvents.

This solubilization process, which therefore can be used to anchor amino acids on solid support through the amino function without protection of the C terminus, was found to be superior over previously reported procedures involving silylation with *N*,*O*-bis(trimethylsilyl)acetamide.[15] After washing with 10% AcOH in dichloromethane to regenerate the free carboxylic acid, the COOH function of **8** was activated with BOP/HOBt/DIEA and the resulting intermediate was readily coupled either with an amine or an amino acid ester. This reaction sequence can be regarded as the starting point in the synthesis of peptides in the N→C direction,[15a] C-terminal modified peptides,[16] or hydantoins.[15b] Each reaction step was monitored by ATR-FT-IR and HRMAS NMR spectroscopy (Figure 4 and Supporting Information).

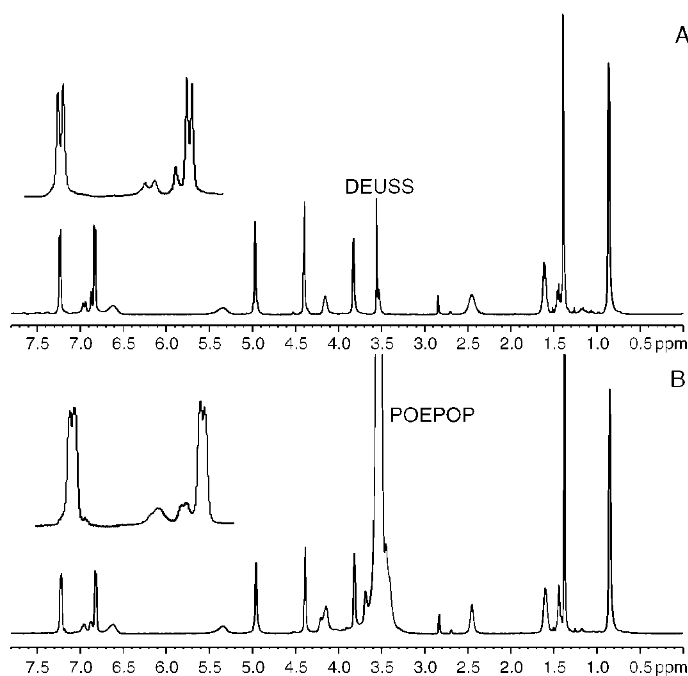


Figure 4. ^{1}H HRMAS spectra of compound **9b** bound to DEUSS and POEPOP, swollen in $CDCl_3$.

The HRMAS spectra display a high quality and a liquid-like resolution, thus allowing easy identification of all the proton resonances of the intermediates and the final products still attached to the solid support. The same reactions were also conducted in parallel on POEPOP resin for comparison. Besides the presence of the intense oxyethylene signal at 3.55 ppm, which renders the HRMAS spectra less clean, their quality was also inferior (Figure 4). The higher resolution of **9b** linked to DEUSS allowed us to measure the coupling constant of the aromatic moiety ($J = 8.11$ Hz) and of δCH_3 proton of Leu residue ($J = 5.25$ Hz) (Figure 4, inset). The fine structure of Leu γCH was visible as a quintuplet at about 1.45 ppm and increased resolution of the NH amide region was also observed.

A further advantage of using DEUSS lies in the reduced quantity necessary for the solid-phase synthesis. Typically, 5 to 10 mg of resin were used as starting material. This amount is sufficient to fill the rotor and to perform a detailed HRMAS NMR characterization. The resin is then fully recovered for the following reaction steps. This is certainly of paramount importance in the optimization of multistep reactions, thus avoiding wasting of material and compensating for the relative high cost of the deuterated reagents used for the preparation of DEUSS. Finally, we would like to anticipate that DEUSS will help in the characterization of molecules that are insoluble or aggregate in solution by linking them to the solid support and conducting HRMAS experiments using the appropriate solvent.

Conclusion

In summary, we have designed and prepared a fully deuterated resin through anionic polymerization by using poly(ethylene glycol) obtained from deuterated ethyleneoxide. We have demonstrated the great potential of DEUSS for the solid-phase synthesis of peptides, synthetic oligomers, and organic molecules, and most importantly for their characterization by HRMAS NMR spectroscopy. On-bead liquidlike NMR spectra of high quality and resolution and devoid of the parasite resin signals could be obtained and readily assigned. In addition, we believe that DEUSS will help to shed light on the role of the solid support regarding the residual NMR linewidth and its origin.[5b,17] Within this context, we are currently studying in more detail the chemicophysical properties of DEUSS.

Experimental Section

Abbreviations: Symbols and abbreviations for amino acids and peptides are in accord with the recommendations of the IUPAC-IUB Commission on Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations used are: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazole-1-yloxytris(dimethylamino)phosphoniumhexafluorophosphate; *t*Bu, *tert*-butyl; DIAD, diisopropylazodicarboxylate; DIEA, diisopropylethylamine; DTT, dithiothreitol; Fmoc, fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methyl morpholine; P_2-Et, 1-ethyl-2,2,4,4-pentakis(dimethylamino)-2λ^5,4λ^5-catenadi(posphazene); PEG, poly(ethylene glycol); -OSu, hydroxysuccinimidyl; MSNT, 1-(mesitylene-

sulfonyl)-3-nitro-1*H*-1,2,4-triazole; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoracetic acid; TIS, triisopropylsilane.

General: All reagents and solvents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were carefully distilled prior to use. POEPOP-NH_2 resin (substitution, 0.64 mmolg^{-1}) was prepared as described by Furrer et al.[4c] RP-HPLC analysis was done on a C_{18} column (5µ, 150×4.6 mm) with a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 5–65% B in 20 min at 1.2 mLmin^{-1} flow rate. Chromatograms were recorded at 214 nm wavelength. MALDI-TOF mass analysis was performed on a linear MALDI-TOF Bruker instrument with α-cyano-4-hydroxycinnamic acid as a matrix. Amino acid analysis was performed on an Applied Biosystem model 130A separation system coupled to an Applied Biosystem model 420A derivatizer.

Preparation of poly([D$_4$]ethylene glycol) (1a): [D$_4$]Ethylene oxide (5 g, 0.1 mol; Cambridge Isotope Laboratories, Andover, MA) was condensed in a graduated dropping funnel maintained in liquid nitrogen. The polymerization vessel, consisting of a three-necked flask equipped with a stirring bar, a reflux condenser, a septum and the dropping funnel containing [D$_4$]ethylene oxide, was assembled on a nitrogen line. The vessel was purged with dry nitrogen by using three evacuation cycles (<0.001 mbar), flame dried with another evacuation/purging cycle and finally cooled to −35°C. Dry THF (approximately 100 mL) was freshly distilled under vacuum into the polymerization flask. Under a gentle nitrogen flow, *t*BuOK (3.5 mL, 1 M in dry THF) was injected by means of a syringe and afterwards [D$_4$]ethylene oxide was added (5.5 mL, 4.79 g, 0.1 mol) in one portion. The polymerization was allowed to continue under nitrogen at room temperature for 5 days and quenched by addition of HCl (0.5 M, 1 mL). The solution was concentrated under vacuum (25 mL), the polymer was precipitated in ice-cold diethyl ether, separated by centrifugation at 4000 rpm for 5 min, washed with diethyl ether (30 mL), and dried under vacuum to give an amorphous slightly brownish solid (4.52 g, 94%). *tert*-Butyl groups were removed by stirring poly([D$_4$]ethylene glycol) (4.52 g, 2.8 mmol) in HCl (4 N, 30 mL) at 50°C for 3 h. The solution was diluted with distilled water (400 mL) and lyophilized. The residue was redissolved in distilled water (40 mL) and the solution neutralized by addition of anhydrous K_2CO_3 (portions of 20 mg). The solution was evaporated to dryness and water was removed by azeotropic evaporation with acetonitrile (2×100 mL). The residue was suspended in acetonitrile (100 mL) and the potassium salt removed by centrifugation at 5000 rpm for 10 min. The supernatant was decanted, evaporated to dryness, and dried under vacuum to yield **1a** (4.18 g, 92%).

Preparation of methyloxirane–poly([D$_4$]ethylene glycol) (1b): Sodium hydride (69 mg, 1.73 mmol, 60% dispersion in mineral oil) was added to a solution of **1a** (1.53 g, 0.96 mmol) in dry THF (2 mL) in a round-bottomed flask at 45°C under argon. The flask was closed with a drying tube filled with $CaCl_2$ to liberate the H_2, and the reaction mixture was stirred at 45°C until the gas evolution was terminated (1.5 h). The drying tube was replaced by a septum with an argon balloon and [D$_5$]epichlorhydrine (159 mg, 1.73 mmol, Aldrich) was added dropwise. The reaction mixture was stirred under argon overnight (16 h) at 45°C. THF was evaporated, and the residue was dried at 50°C under vacuum and redissolved in dry acetonitrile (10 mL) at 50°C. After cooling to room temperature the precipitated sodium salt was removed by centrifugation at 5000 rpm for 20 min and washed with acetonitrile (2×3 mL). The combined supernatants were extracted with heptane (2×10 mL) to remove mineral oil and evaporated. The residue was dried under high vacuum at 50°C to yield **1b** (1.47 g, 90%).

Preparation of DEUSS (1): Mixture **1b** (1.47 g, 0.92 mmol) was melted at 50°C under argon, *t*BuOK (52 mg, 0.46 mmol) was added, and the reaction mixture stirred under argon at 50°C for 30 min. The flask was placed into a preheated oil bath (130°C), where the reaction was allowed to proceed under argon at 130°C overnight (16 h). The resin was treated with HCl (4 M) for 3.5 h at room temperature and extensively washed with water. The loading was calculated by reaction of a weighted amount of resin with Fmoc-Gly-OH (3 equiv), MSNT (3 equiv) and *N*-methylimidazole (2.5 equiv) in dry CH_2Cl_2 for 3 h. After repeating the reaction twice, Fmoc was removed with 25% piperidine in DMF and recovered for the determination of the UV absorbance at 300 nm. Loading was about 0.55 mmolg^{-1}.

Synthesis of DEUSS-NH$_2$ (2): This resin was prepared from DEUSS **1** by the procedure described by Furrer et al.[4c] The loading was calculated by reaction of a weighted amount of resin with Fmoc-Ahx-OH (5 equiv), BOP (5 equiv), HOBt (5 equiv) and DIEA (15 equiv) in DMF for 2 h. After repeating the reaction twice, Fmoc was removed with 25% piperidine in DMF and recovered for the determination of the UV absorbance at 300 nm. Loading was about 0.50 mmolg^{-1}.

Synthesis of 4-(*tert*-butyldimethylsilyloxymethyl)phenoxyacetic acid: 4-(Hydroxymethyl)phenoxyacetic acid (1.822 g, 10 mmol) and imidazole (3.007 g, 40 mmol) were suspended in dry CH_2Cl_2 (20 mL). *tert*-Butyldimethylsilyl chloride (2.723 g, 20 mmol) was solubilized in dry CH_2Cl_2 (10 mL) and dropped to the suspension over a period of 45 min. The mixture was stirred for 48 h and the solvent evaporated. The oily residue was dissolved in AcOEt and washed with $KHSO_4$ (1 N) and water. The organic phase was dried over Na_2SO_4 and evaporated recovering the expected compound as a white powder. Yield: 78%; ^{1}H NMR (CDCl$_3$): δ=10.23 (s, 1H), 7.26 (d, 2H), 6.89 (d, 2H), 4.69 (s, 2H), 4.67 (s, 2H), 0.94 (s, 9H), 0.10 ppm (s, 6H); ^{13}C NMR (CDCl$_3$): δ=174.38, 156.48, 134.96, 127.64, 114.44, 64.90, 64.53, 25.93, 18.40, −5.24 ppm.

Synthesis of DEUSS-4-(hydroxymethyl)phenoxyacetamide (4): A solution of 4-(*tert*-butyldimethylsilyloxymethyl)phenoxyacetic acid (89 mg, 10 equiv), BOP (133 mg, 10 equiv) and HOBt (40 mg, 10 equiv) in DMF (3.0 mL) was added to the DEUSS-NH$_2$ **2** resin (50 mg, 25 µmol), followed by DIEA (153 µL, 30 equiv). The mixture was shaken 3.5 h at room temperature affording resin **3**. Removal of the TBDMS group was accomplished by treating the resin for 1 h with a solution of TBAF (1 M, 15 equiv) in THF. Following extensive washing with CH_2Cl_2, MeOH, water, the resin was finally washed with a solution of 5% AcOH in CH_2Cl_2 to transform the tetrabutylammonium salt into the free hydroxy group, thus obtaining DEUSS **4**.

Peptide synthesis: The sequence of the peptide, corresponding to the VP1 region 141–159 of FMDV (variant USA), used in this study is ^{141}GSGVRGDFGSLAPRVARQL^{159}. An additional 6-aminohexanoic acid residue (Ahx) was added to the C-terminal part of the peptide in order to have a spacer between the peptide and the resin. The N terminus of the peptide was acetylated. The synthesis of the FMDV peptide bound to DEUSS **2** (12 µmol scale) was performed on a multichannel peptide synthesizer by means of the standard Fmoc/*t*Bu strategy.[18] The side-chain protecting groups were removed by using a mixture of TFA/TIS/DTT/water 88:2:5:5. The peptide was characterized by amino acid analysis after hydrolysis in HCl (6 N) for 24 h at 110°C.

Synthesis of oligoureas: Assembly of ureas **6a–c** was carried out on a 1.25 µmol scale starting from DEUSS **2** and using succinimidyl-2-[(*tert*-butoxycarbonyl)amino]-2-substituted-ethyl carbamates with side chains of Phe and Val as monomers.[19] For each coupling step, a solution of the appropriate carbamate (5 equiv) in DMF (300 µL) and DIEA (5 equiv) were added subsequently to the resin. The suspension was shaken for 2 h at room temperature and a double coupling was performed systematically. Monitoring of the coupling reaction was performed with the Kaiser test.[20] At the end of the reaction, the resin was washed with DMF (6× 300 µL). The Boc group was removed by treatment with TFA (300 µL, 2×5 min) and the resin was washed with CH_2Cl_2, *i*PrOH, and DMF. The final ureas on solid support were washed with CH_2Cl_2 and diethyl ether, and dried under vacuum at 50°C for 12 h.

Synthesis of small organic molecules 9: DEUSS **4** (10 mg, 5 µmol) was swollen in dry CH_2Cl_2 (1 mL). NMM (5 equiv) was added and the mixture was cooled at 0°C. 4-Nitrophenyl chloroformate (5 equiv) was subsequently added and the mixture was stirred at room temperature for 16 h. The solution was removed, and the resin washed with CH_2Cl_2 and diethyl ether, and dried under vacuum at 50°C. A suspension of Leu (10 equiv) was prepared in dry THF (1 mL), followed by the addition of phosphazene base P_2-Et (10 equiv). The mixture was added to DEUSS **7** (11 mg, 5 mmol) previously swollen in THF (1 mL). The resin was stirred at room temperature for 16 h, washed with THF, water, 5% AcOH in CH_2Cl_2, CH_2Cl_2, and diethyl ether, and dried under vacuum at 50°C. DEUSS **8** (11 mg, 5 mmol) was swollen in DMF (1 mL) and a mixture of BOP/HOBt (5 equiv) and R-NH$_2$ (5 equiv) in DMF (1 mL) was added followed by DIEA (10 equiv). The suspension was shaken at room temperature for 1.5 h. The coupling was repeated twice under the same conditions. The resin was washed with DMF, CH_2Cl_2, and diethyl ether, and

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dried under vacuum at 50°C. The same reaction was conducted in parallel on POEPOP resin functionalized with the same linker.

NMR spectroscopy: The identification of amino acid spin systems and sequential assignment were made by using a combination of HRMAS TOCSY,[21] NOESY,[22] and HSQC[23] experiments. HRMAS 1D and 2D NMR spectra were obtained on Bruker Avance 500 MHz spectrometer equipped with for 4 mm $^{1}H/^{13}C/^{15}N/^{2}H$ HRMAS gradient probe. The samples (3–5 mg) were packed into a 4 mm HRMAS rotor and solvents were added to the resin directly inside the rotor. Samples were spun at 6–8 kHz. The spectra were acquired at 300 K and referenced to the peak of the solvent. All 2D spectra were recorded in pure phase mode by using the States-TPPI method. Homonuclear spectra were recorded with 2048 data points in $t2$ and 256 increments in $t1$. Typically 16 scans per increment were accumulated. A spectral width of 5482.46 Hz was used for the proton. TOCSY data were recorded with a DIPSI-2[24] sequence of 50 or 60 ms. Through-space dipolar connectivities were obtained from NOESY spectra by using mixing times from 150 to 300 ms. $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HSQC spectra were recorded with 2048 data points in $t2$ and 256 increments in $t1$. The number of scans accumulated for $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HSQC were 64. Sweep widths for ^{1}H and ^{13}C dimensions were 5482.46 Hz and 22640.04 Hz, respectively, while spectral widths for ^{1}H and ^{15}N dimensions were 5482.46 and 4055.05 Hz, respectively. The samples were swollen in $CDCl_3$, $[D_7]DMF$, $[D_6]DMSO$, and CD_3CN. MAS 1D NMR spectra were obtained on Bruker Avance 500 MHz spectrometer equipped with for 4 mm HXMAS4 probe. The resins were packed into a 4 mm MAS rotor. The samples were spun at 15 kHz and the spectra were acquired at 300 K.

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