ULTRA Loaded Resins Based on the Cross-Linking of Linear Poly(ethylene imine). Improving the Atom Economy of Polymer-Supported Chemistry**

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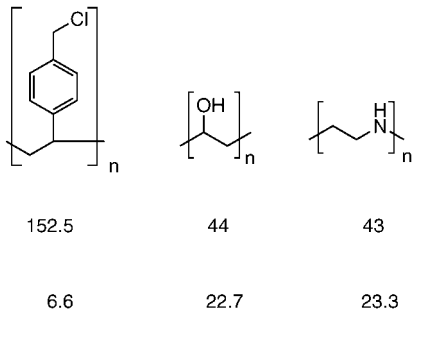
Employing insoluble polymer supports for chemical transformations belongs to the most important and far-reaching innovations in organic chemistry during the last decades.[1] The method had enormous impact as it not only revolutionized the synthesis of peptides, oligonucleotides, heterocycles, and other molecule classes, but also triggered the evolution of combinatorial methods in chemistry and biochemistry.[2] In recent years, polymer supports have found broad application in solid-supported solution-phase syntheses as well.[3]

To date the vast majority of solid-supported chemistry is conducted on cross-linked, swellable polymeric gels; most often polystyrene gels are employed.[4] If increased polarity of the resin is required, polystyrene grafted with poly(ethylene glycol) (PEG) is preferred.[5] By cross-linking of PEG chains, biocompatible supports are obtained that can be penetrated by small to medium-sized biological macromolecules; these polymers are of great interest for biological assays.[6]

One major drawback of the current methods of solid-supported chemistry with conventional resins[8] is the low atom economy[7] in comparison to solution-phase synthesis; this disadvantage excludes solid-supported methods from many resource- and cost-sensitive applications such as scale-up projects. Polystyrene resins are further restricted in the choice of solvents, thermal and chemical stability, and extensive adsorption of reagents.[9]

We anticipated that a novel class of resins constructed of low molecular-weight monomers with efficient functionalization sites at each monomer would greatly enhance the resin capacity. Poly(ethylene imines) (PEI)[10] possess a loading of 23 mmol g^{-1} amines, thus ULTRA resins based on the cross-linking of PEI could permit solid-phase chemistry with significantly higher atom economy than conventional polymers (Scheme 1). Linear poly(ethylene imine) (**1**) was selected as the polymeric starting material, which allowed control over the degree of cross-linking and the degree of amine substitution in the resin product. ULTRA resins were also prepared from tetraethylene pentamine, a well-defined, linear-PEI fraction.



$M_{monomer}$ [g mol^{-1}]	152.5	44	43
loading [mmol g^{-1}]	6.6	22.7	23.3

Scheme 1. Potential building blocks for ULTRA loaded resins based on poly-(4-chloromethyl styrene), poly(vinyl alcohol) (PVA), and poly-(ethylene imine) (PEI).

Polyaldehydes, polyacids, and polyvalent alkylating agents were investigated for cross-linking. Polyaldehydes, which furnish the resin structure through a thermodynamically controlled equilibrium reaction, were found to be superior, allowing the preparation of resins with robust methods from inexpensive and easily available precursors. Reducing the condensation product of linear poly(ethylene imine) (**1**) with terephthalic dialdehyde (**2**) afforded the chemically, mechanically, and thermally robust ULTRA resin (**3**; Scheme 2).[11] A support prepared from a molar ratio of 1.67 dialdehydes per PEI chain was selected for detailed characterization and synthetic studies. Resin micropellets of a defined size range were generated by polymer extrusion in the swollen state, followed by sieving.

Whereas polymeric salt **4** swelled only slightly in organic solvents, in water it extended to the remarkable gel volume of 180 mL g^{-1},[12] larger than any of the commercially available resins and suggesting the possible penetration of the resin by large molecules. The free-amine resin **3**, as was the case with the chemically derivatized resins **5**–**10** and **12**–**14**, displayed swelling volumes between 3–8 mL g^{-1} in various solvents—useful for polymer-supported chemistry (Scheme 3). Spectroscopic resin analysis was conducted by FT-ATR-IR, ^{13}C suspension NMR, and ^{1}H magic-angle-spinning (MAS) suspension NMR spectroscopy, which revealed the absence of aldehyde, alcohol, and primary amine functionalities in the resin network of **3**; this indicates complete cross-linking and reduction of the imine groups. The ratio of PEI to the cross-linker (1.55) was derived from the integrated ^{1}H NMR spectrum and was consistent with the starting mixture; the ratio of secondary to tertiary amines in **3** was calculated from the ratio of PEI to cross-linker to be 8:1.

Accessibility of the secondary amines in ULTRA resin **3**—a crucial prerequisite for successful polymer-supported chemistry—was investigated by acylation (**5** and **10**), nitrosylation (**6**), and alkylation reactions (**7**–**9**; Scheme 3). Efficient reactions were indicated by the complete removal of the band arising from the NH group in the IR spectrum, consistent weight increase of the resins, and by elemental analysis.

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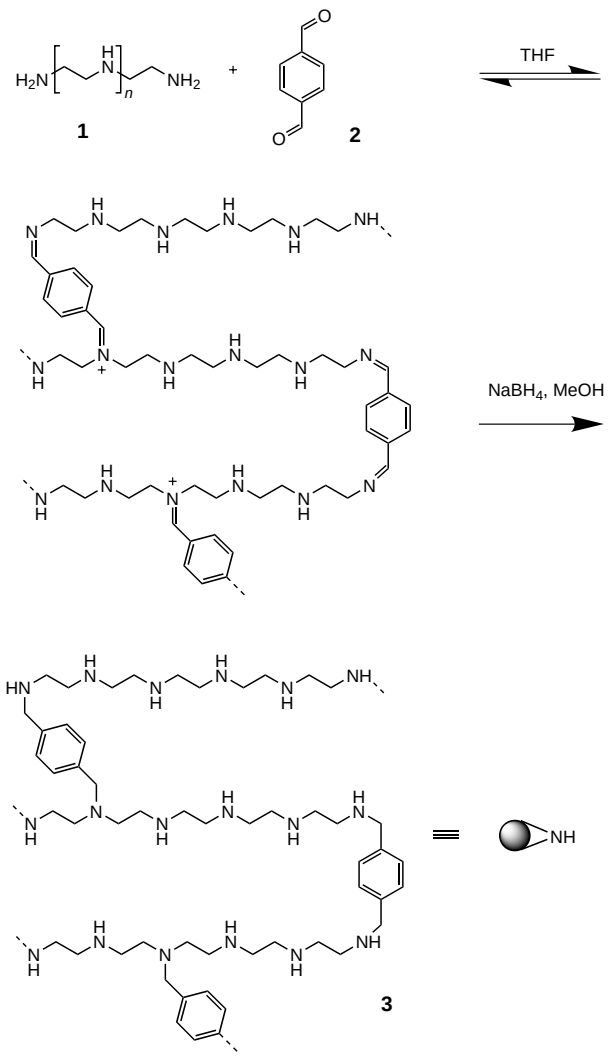


Scheme 2. Preparation of ULTRA resin **3** from **1** and **2**.

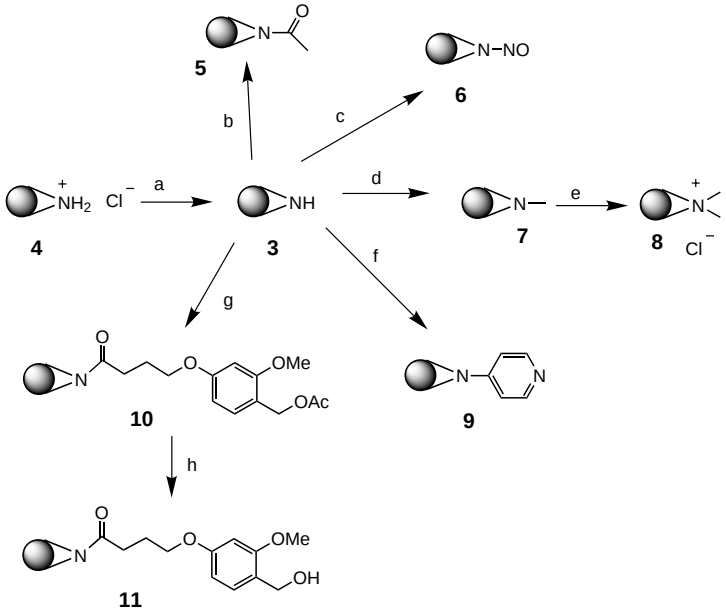
ULTRA resins were employed for the preparation of polymeric reagents as well as for solid-phase synthesis. ULTRA resin **4** can be directly employed as a polymeric base with a loading of 15.2 mmol g^{-1}. Following reductive amination with formaldehyde, resin **7**, containing 13.2 mmol g^{-1} tertiary amines, was obtained. ULTRA resin **8** for ion exchange was prepared by alkylation of **7** with methyl iodide; the maximum loading of the resin with chloride was 8 mmol g^{-1}, which corresponds to a chlorine content of 28%. The acylation catalyst **9** could be constructed from **3** by microwave-assisted synthesis at 220 °C[17].

An ULTRA resin for solid-phase synthesis (**10**) was constructed by coupling resin **3** with 4-(4′-acetoxymethyl-3′-methoxy-phenoxy)butyrate. Following complete deacetylation (detected by IR), resin **12**, with a loading[15] of 2.5 mmol g^{-1}, was employed in the synthesis of heterocycles as well as peptides. Pyrazole carboxylic acid **13** was prepared on **11** using a procedure[13] established for Wang polystyrene without modification (Scheme 4). After three steps that were monitored by IR, 8.8 mg of product **13** were obtained from 5 mg of the starting ULTRA resin **3** (80% purity of the crude product, 65% yield following chromatography).

To investigate limitations in product size, several peptides of various lengths were synthesized on ULTRA resin **11**. Peptides with 7, 9, 13, and 19 amino acids were prepared on a synthesis robot employing Fmoc-protected amino acids and carbodiimide–hydroxybenzotriazole activation (0.25 M) without detection of deletion products. To illustrate the remarkable economy of the ULTRA resin, in this example 3.4 mg of the starting resin **4** sufficed for the synthesis of 42 mg resin with fully protected tridecapeptide. The crude product was obtained by trituration with diethyl ether in excellent purity and yield (90% purity in the crude product, 78%, 13.1 mg, following preparative HPLC).[16]

In summary, ULTRA resins based on the cross-linking of linear PEI have been realized as a novel support concept with high potential for polymer-supported synthesis in solution as well as for solid-phase synthesis. In contrast to any of the currently available supports, which are obtained by radical, anionic, or cationic polymerizations, ULTRA resin **3** is formed under thermodynamic control in an equilibrating reaction system with potentially significant implications for polymer structure and properties.

ULTRA resins were prepared with extremely high loading compared to the standard resins in use today. The secondary amine groups of the resin were very accessible to various efficient derivatizations and even larger product molecules could be assembled successfully in the resin interior. Thus, the resins allow solid-supported chemistry with greatly improved atom economy and could be a significant contribution to the efficient scale-up of polymer-supported syntheses. Extended



Scheme 3. Accessibility of ULTRA resin **3** investigated by chemical derivatization: a) 1. 2 M NaOH, 2. triethylamine, DMF; b) Ac_2O, pyridine; c) $NaNO_2$, HOAc, water; d) CH_2O, $NaCNBH_3$; e) 1. MeI, 2. HCl, water; f) 4-chloropyridinium chloride, diisopropylethylamine (DIPEA), DMF, 220 °C, 10 min, microwave irradiation; g) 4-(4-acetoxymethyl-3-methoxyphenoxy)butyric acid, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), DIPEA, DMF (2×); h) NaOMe, MeOH.

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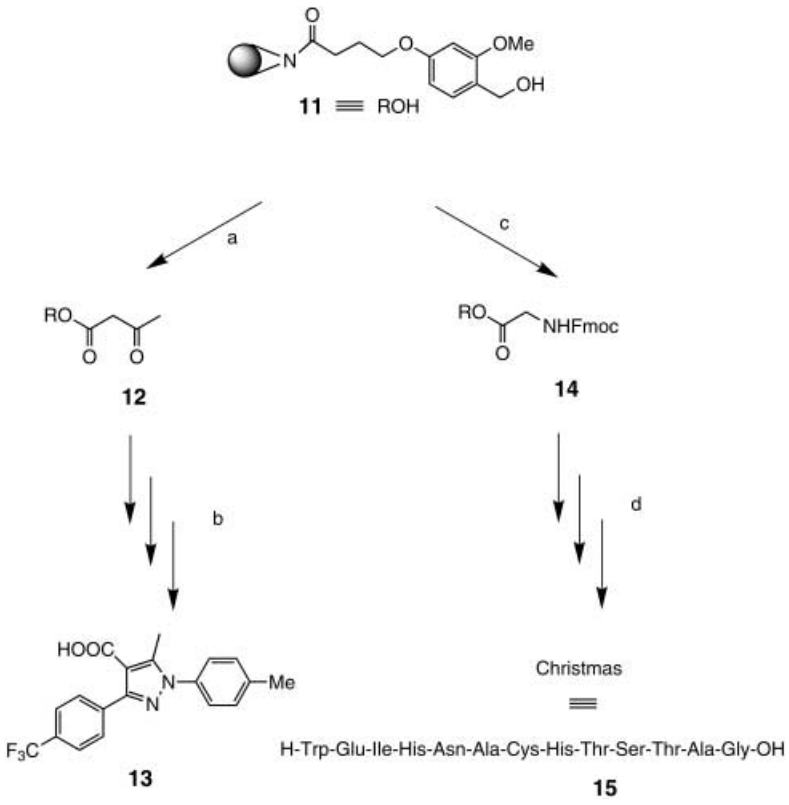

Scheme 4. Synthesis of pyrazole carboxylic acids and peptides on linker-functionalized ULTRA resin **11**; a) diketene, *N*,*N*-dimethyl-4-aminopyridine (DMAP);[12] b) 1. 3 equiv 4-trifluoromethyl benzaldehyde, DIPEA, *N*-methylpyrrolidin-2-one (NMP), 16 h, 2. 3 equiv 4-methylphenylhydrazinium hydrochloride, 3. CH_2Cl_2/HOAc, 30 min, 4. TFA/water 95:5. c) Fmoc-Gly-OH, 2-mesitylenesulfonyl-3-nitro-1*H*-1,2,4-triazole (MSNT), *N*-methyl-imidazole, CH_2Cl_2, 2 × 2 h; d) 1. amino acid coupling: protected Fmoc amino acid, *N*,*N*-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), DMF, 2. amine deprotection: 20% piperidine, DMF, 3. TFA, water (5%), triisopropylsilane (2.5%), ethanedithiol (2.5%).

synthetic investigations will scrutinize further the potential of PEI-based ULTRA resins as well as variations of this concept starting from alternative starting primary polymers and cross-linkers.

Experimental Section

Linear poly(ethylene imine) (**1**): Elemental analysis: C 53.1, H 11.9, N 34.5. C/N = 1.54. The degree of polymerization (n) was calculated from the C/N ratio ($n = 0.583/(N/C-0.583)$) and was 8.74 corresponding to an average molecular mass of $M_n = 393.5\ g\,mol^{-1}$ ($M_n = 43.07n + 17.03$). M_n was specified by the supplier as $423\ g\,mol^{-1}$.

Preparation of ULTRA resin **3**: **1** (4.17 g, Aldrich, $M_n = 393.5\ g\,mol^{-1}$, $n =$ 8.74, 10.6 mmol, 103.2 mmol amine groups) was dissolved in THF (5 mL) in a 50 mL round-bottomed flask with magnetic stirring. **2** (2.38 g, 17.7 mmol) was added rapidly in THF (17.5 mL solution). After 30 s the flask had warmed to about 40 °C, after 4 min the stirrer bar ceased rotating because of increasing viscosity, after 6 min it remained stationary. The initially transparent polymer gel became opaque after 10 min. 3 h after initiation of the reaction the polymer was granulized in a mortar and reduced using $NaBH_4$ (1 g) in MeOH (100 mL) for 1 h. After quenching with water (100 mL), the polymer was filtered, washed (1M HCl, water, THF), and extruded through a metal sieve (400 μm pores) with the addition of MeOH. The resin was washed again (THF, CH_2Cl_2) on a ceramic filter (P3, ca. 40 μm max. pore size) and dried in vacuo over P_2O_5 yielding resin micropellets of a defined size range (4.8 g). The hydrochloride resin **3** was transformed to the free-amine resin **4** by swelling in 2M NaOH, followed by washing with water, 20% triethylamine in DMF, DMF, THF, and CH_2Cl_2.

3: ^{1}H NMR (250 MHz, D_2O, HR-MAS with 4500 Hz rotation): $\delta = 2.7$–3.5 (m, PEI-CH_2, relative integration 100), 4.31 (bs, N-CH_2-aryl, 14.6), 7.4–7.6 ppm (bs, aryl-H, 17.7). FT-ATR-IR: $\tilde{\nu} = 1443$, 1590, 2768, 2950, 3371 cm^{-1}. Elemental analysis: C 41.9, H 8.0, N 14.5 ($10.4\ mmol\,g^{-1}$), Cl 23.3.

4: ^{13}C NMR (62.9 MHz, D_2O, DEPT-135): $\delta = 49.7$ (CH_2), 54.5 (CH_2), 131.4 ppm (CH). FT-ATR-IR: $\tilde{\nu} = 1110$, 1164, 1331, 1392, 1554, 2826, 2941, 3291 cm^{-1}. Elemental analysis: C 61.8, H 10.5, N 21.3 ($15.2\ mmol\,g^{-1}$).

13: ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.36$ (s, 3H, PhMe), 2.52 (s, 3H, 5-Me), 7.25 (dd, 4H, 1-Ph), 7.60, 7.78 (2 d, 4H, 3-Ph). Calcd ($C_{19}H_{15}F_3N_2O_2$): 360.33 Da, found (ESI-MS, positive mode): 361.0 m/z: (ESI-MS, negative mode): 359.0 m/z.

15: Calcd ($C_{60}H_{87}N_{19}O_{20}S_1$): 1425.61 Da, found (ESI-MS, positive mode): 1426.6 m/z.

Spectra of the ULTRA resins are provided in the Supporting Information.

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[1] a) R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149–2151; b) F. Zaragoza Dörwald, *Solid-Phase Synthesis*, Wiley-VCH, Weinheim, **2000**; c) D. Hudson, *J. Comb. Chem.* **1999**, *1*, 333–360, 403–457.

[2] a) *Combinatorial chemistry—Synthesis, analysis, screening* (Ed.: G. Jung), Wiley-VCH, Weinheim, **1999**; b) *Combinatorial peptide and nonpeptide libraries*, (Ed.: G. Jung), VCH, Weinheim, **1996**.

[3] a) S. V. Ley, I. R. Baxendale, R. M. Bream, P. S. Jackson, A. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer, S. J. Taylor, *J. Chem. Soc. Perkin Trans. 1* **2000**, *23*, 3815–4195; b) A. Kirschning, H. Monenschein, R. Wittenberg, *Angew. Chem.* **2001**, *113*, 670–701; *Angew. Chem. Int. Ed.* **2001**, *40*, 650–679; c) J. Rademann, J. Smerdka, G. Jung, P. Grosche, D. Schmid, *Angew. Chem.* **2001**, *113*, 390–393; *Angew. Chem. Int. Ed.* **2001**, *40*, 381–385; d) J. Rademann, W. Kraas, B. Dörner, *Nachr. Chem.* **2000**, *48*, 280–283; e) S. Weik, G. Nicholson, G. Jung, J. Rademann, *Angew. Chem.* **2001**, *113*, 1489–1492; *Angew. Chem. Int. Ed.* **2001**, *40*, 1436–1439; f) G. Sorg, A. Mengel, G. Jung, J. Rademann, *Angew. Chem.* **2001**, *113*, 4532–4535; *Angew. Chem. Int. Ed.* **2001**, *40*, 4395–4397.

[4] D. C. Sherrington, *Chem. Commun.* **1998**, 2275–2286.

[5] W. Rapp, L. Zhang, R. Häbish, E. Bayer in *Peptides 1988, Proc. Eur. Pept. Symp.* (Eds.: G. Jung, E. Bayer), Walter de Gruyter, Berlin, **1989**, pp. 199–201.

[6] a) M. Meldal, *Tetrahedron Lett.* **1992**, *33*, 3077–3080; b) J. Rademann, M. Meldal, K. Bock, *Chem. Eur. J.* **1999**, *5*, 1218–1225; c) J. Rademann, M. Grøtli, M. Meldal, K. Bock, *J. Am. Chem. Soc.* **1999**, *121*, 5459–5465; d) T. Groth, M. Grøtli, L. P. Miranda, W. D. Lubell, M. Meldal, *J. Chem. Soc. Perkin Trans. 1* **2000**, 4258–4264.

[7] B. M. Trost, *Angew. Chem.* **1991**, *107*, 285–307; *Angew. Chem. Int. Ed.* **1991**, *30*, 214–234.

[8] Conventional resins possess loadings between 0.2–1 $mmol\,g^{-1}$. Loadings can be increased by dendrimer synthesis on polystyrene supports, or by graft polymerization: V. Swali, N. J. Wells, G. J. Langley, M. Bradley, *J. Org. Chem.* **1997**, *62*, 4902–4903; C. W. Lindsey, J. C. Hodges, D. M. Leonard, G. F. Filzen, *J. Comb. Chem.* **2000**, *2*, 550–559.

[9] J. Rademann, M. Barth, R. Brock, H.-J. Egelhaaf, G. Jung, *Chem. Eur. J.* **2001**, *7*, 3884–3889.

[10] Branched PEI is employed as a process chemical in the paper industry and is produced by cationic ring-opening polymerization from aziridine in kilotonne amounts. Branched poly(ethylene imine) has been cross-linked for use in ion exchange and for enzyme immobilization: a) E. J. Shepherd, J. A. Kitchener, *J. Chem. Soc.* **1957**, 86–92; b) G. Manecke, S. Heydolph, *Makromol. Chem.* **1981**, *182*, 2641–2657.

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[11] Stability of the resin was investigated under strongly basic (10% NaOH) as well as strongly acidic reaction conditions (37% HCl) and at 220°C (DMF, microwave irradiation) without observing any modification of the resin structure and morphology.
[12] Swelling volumes were determined by compressing a swollen resin sample in a 5-mL syringe with a weight of 2 kg and measuring the resin volume after pressure release.
[13] P. Grosche, A. Höltzel, T. B. Walk, A. W. Trautwein, G. Jung, *Synthesis* **1999**, 1961 – 1970.
[14] B. Blankemeyer-Menge, M. Nimtz, R. Frank, *Tetrahedron Lett.* **1990**, *31*, 1701 – 1704.
[15] Loading of hydroxy resins were determined by coupling Fmoc-Gly-OH employing the method described in ref. [14], followed by Fmoc-cleavage and spectrophotometric quantification at 267, 289, and 301 nm.
[16] The efficiency of the acylation steps on the ULTRA resins was highlighted by the structure of the main by-product (6%), which was not a deletion product, but **15** + *tert*-butyl, an incompletely deprotected 13mer-peptide.
[17] Microwave-assisted syntheses were performed on a SmithSynthesizer from Personal Chemistry AB, Uppsala, Sweden.

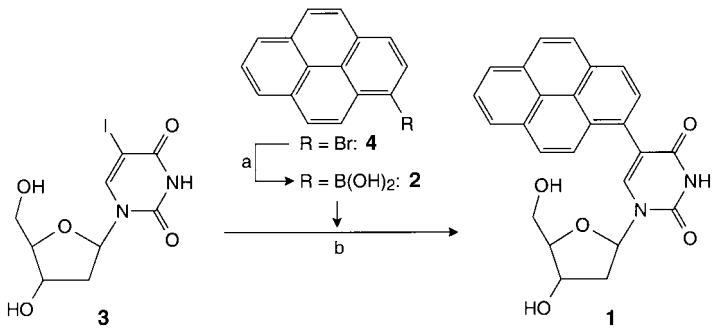
A Model Nucleoside for Electron Injection into DNA: 5-Pyrenyl-2′-Deoxyribose**

Nicole Amann, Evgeni Pandurski, Torsten Fiebig,* and Hans-Achim Wagenknecht*

Charge-transfer processes through DNA have been studied intensively in the past 15 years.[1] It is important to emphasize, that in most of these experiments oxidative hole transfer has been observed. On the other hand, reductive electron transfer (ET) is currently used extensively in DNA chip technology[2] and DNA nanotechnology[3] without an understanding of the mechanism of this type of charge-transfer. Recently, Carell and co-workers described the repair of a thymine–thymine dimer by a distant flavine derivative, which was incorporated into the DNA as an artificial base.[4] Despite the fact that spectroscopic measurements with this system have not been published, the cleavage of the thymine–thymine dimer was interpreted as the chemical result of a reductive ET through the DNA base stack. To date, suitable DNA assays for the time-resolved spectroscopic investigation of reductive ET through DNA are elusive.

We present herein the synthesis and pH-dependent spectroscopic investigation of the ET in the model nucleoside 5-pyrenyl-2′-deoxyuridine (Py-dU, **1**). Pyrene derivatives have been used previously as artificial DNA bases by Kool et al.[5] We chose a different approach and attached the pyrenyl group covalently to the uracil or thymine nucleobases. Excitation of the pyrene moiety at 340 nm leads to an intramolecular ET, which yields the corresponding uracil radical anion and the pyrenyl radical cation ($Py^{\bullet+}$-$dU^{\bullet-}$). This charge-transfer assignment has been proven previously by Netzel et al.[6] Based on the reduction potential for the $Py^{\bullet+}$/Py redox couple of 1.52 V (vs. normal hydrogen electrode)[7] and $E_{00} = 3.25$ eV,[6] the driving force ΔG of this ET process has a maximum value of −0.5 eV based on the potential of −1.2 V for the $dU/dU^{\bullet-}$ redox couple.[8] However, this value of $|\Delta G|$ seems to be too large with respect to a recent femtosecond time-resolved study on the reduction of thymine, which suggests a potential of approximately −1.8 V for the $dT/dT^{\bullet-}$ redox couple.[9] Based on steady-state fluorescence spectroscopy and nanosecond fluorescence lifetime measurements, it was proposed that ET from the photoexcited pyrene to the uracil moiety should be more favorable in MeOH than in MeCN because of a proton-coupled ET process.[6] However, this hypothesis is in disagreement with the proposed pK_a value of 6.9 for the protonated uracil radical anion $dU(H)^{\bullet}$ reported by Steenken.[10] Therefore, the prononated radical $dU(H)^{\bullet}$ represents a stronger acid than MeOH and cannot be protonated by this solvent. To elucidate the possibility of a proton-coupled ET in more detail, we chose water at different pH values and measured the steady-state fluorescence and time-resolved transient absorption spectra. An understanding of the protonation dynamics of radical anions of DNA bases is crucial to understand both ET and hole transfer through DNA. Moreover, to investigate the competition between ET to adjacent DNA bases and protonation by surrounding water molecules and/or hydrogen-bonded bases, it is particularly important to evaluate the applicability of **1** as an electron injector into DNA.

The nucleoside **1** was prepared by using the palladium-catalyzed Suzuki–Miyaura type cross coupling[11] of pyren-1-ylboronic acid (**2**) to 5-iodo-2′-deoxyuridine (**3**) in a good yield of 79% (Scheme 1). Suzuki–Miyaura type couplings



Scheme 1. Synthesis of **1**: a) 1) *n*BuLi (1.1 equiv), Et_2O, 0°C, 30 min; 2) $B(OCH_3)_3$ (5.0 equiv), −78°C, 6 h, then room temperature, 20 h; 3) H_3O^+, RT, 3 h, 73%; b) **2** (1.0 equiv), $[Pd(PPh_3)_4]$ (0.1 equiv), NaOH (20 equiv), THF/MeOH/H_2O 2:1:2, reflux, 20 h, 79%.

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