Communications

"Click" Chemistry by Microcontact Printing**

Dorota I. Rozkiewicz, Dominik Jańczewski,
Willem Verboom, Bart Jan Ravoo,* and
David N. Reinhoudt*

Microcontact printing (μCP) is commonly used to pattern self-assembled monolayers (SAMs) as etch resists or chemical templates on gold and silicon oxide substrates.[1] However, μCP can also be used for chemical synthesis on gold and silicon oxide in the nanoscale confinement between stamp and substrate. Amines can be printed onto reactive anhydride SAMs.[2] Peptides can be synthesized by printing N-protected amino acids onto an amine SAM.[3] It has been proposed that the confinement of the ink at the interface between the elastomeric stamp and the SAM, in combination with the preorganization of the reactants in the monolayer, facilitates the formation of covalent peptide bonds.[3] Recently, we demonstrated imine formation through the μCP of amines on aldehyde SAMs[4] and applied this chemistry to directing the immobilization of cytophilic proteins.[5] Herein, we present a Huisgen 1,3-dipolar cycloaddition, as a representative example of the Sharpless "click" chemistry,[6] induced without any catalyst by μCP of acetylenes onto azido-terminated SAMs on silicon oxide substrates.

Click chemistry includes a range of reactions that proceed in high yield under ambient conditions, preferably in water, with regioselectivity and a broad tolerance of functional groups.[6] The Cu-catalyzed 1,3-dipolar cycloaddition reaction of azides and acetylenes is known as the "cream of the crop" of all click reactions.[7] This cycloaddition is irreversible and proceeds in quantitative yield without any side products in many solvents, including water. Acetylenes and azides are stable starting materials that do not react among themselves. Azides are known for their ease of introduction, and both azides and acetylenes are tolerant of many other functionalities. The triazole group is a thermally and hydrolytically stable, conjugated linkage. Increasingly, click chemistry is used for the preparation of biological conjugates and the immobilization of biomacromolecules.[8] Triazole formation

[*] D. I. Rozkiewicz, Dr. D. Jańczewski, Dr. W. Verboom, Dr. B. J. Ravoo,
    Prof. Dr. D. N. Reinhoudt
    Laboratory of Supramolecular Chemistry and Technology
    MESA+ Institute for Nanotechnology
    University of Twente
    P.O. Box 217, 7500 AE Enschede (The Netherlands)
    Fax: (+31) 53-489-4645
    E-mail: b.j.ravoo@utwente.nl
           d.n.reinhoudt@utwente.nl

on surfaces was applied for the modification of SAMs on gold substrates,[9] SAMs on silicon oxide,[10] carbon nanotubes,[11] and polymer adhesives.[12] Azido-terminated monolayers were used for the immobilization of substituted acetylenes (including ferrocene and oligonucleotides[9,10,13]) by triazole linkage.

The majority of Huisgen reactions—in particular those that involve electron-rich acetylenes—need a Cu^I catalyst, which may accelerate the reaction by a factor of 10^7. The Cu^I catalyst is usually generated in situ from Cu^{II} by an excess of reducing agent, such as sodium ascorbate.[7] Here, we show that the high local concentration of reagents in the contact area of the elastomeric stamp and the monolayer surface is sufficient to obtain complete conversion within minutes of contact time, even without a Cu catalyst. In particular, for the immobilization of biomolecules it is advantageous to exclude toxic Cu catalysts.

Azido-terminated SAMs on silicon oxide were prepared by the substitution of a bromo-terminated monolayer with NaN_3.[14] The bromo-terminated SAM was obtained by the immersion of a 1×1-cm piece of silicon wafer (cleaned and activated with piranha solution) in a solution of 11-bromoundecyltrichlorosilane in toluene (0.1 %, v/v) for 20 min at 20°C. A saturated solution of NaN_3 in DMF (48 h at 70°C) was used to substitute the bromine for the azido group.[14] The substrate was rinsed with MilliQ water and ethanol and dried with nitrogen. The preparation of the azido-terminated SAMs and the subsequent click chemistry by µCP of acetylenes are outlined in Scheme 1.

Figure 1. Tapping-mode AFM image (50×50 µm) and line section of a 3×5-µm line pattern obtained by printing 1-octadecyne onto azido-terminated SAM **2** on a silicon oxide substrate.

Scheme 1. Click chemistry by µCP. **1**: Bromo-terminated SAM on a Si/SiO₂ substrate. **2**: Azido-terminated SAM. **3**: Triazole SAM after µCP of 1-octadecyne onto azido-terminated SAM.

As a first illustration of the potential of µCP of acetylenes on azido-terminated SAMs on a silicon substrate, 1-octadecyne was printed onto azido SAM **2** with a 1×1-cm poly(dimethylsiloxane) (PDMS) stamp with line features of 3×5 µm. The stamp was inked in a solution of 1-octadecyne in ethanol (5 mM) for 1 min, dried with nitrogen, and brought into contact with the substrate SAM **2** for 15 min with a load of 35 g to ensure good contact and constant applied load during the entire printing time. After extensive rinsing with ethanol, the substrate was imaged with atomic force microscopy (AFM). Figure 1 shows the pattern made by printing 1-octadecyne on substrate **2**. Evidently, the 3×5-µm line features of the stamp are reproduced faithfully on the
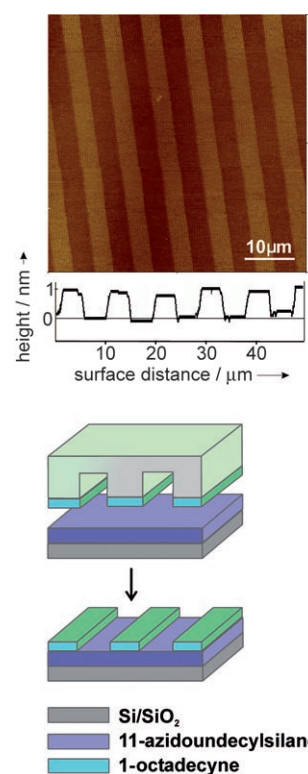
substrate. The average height of the 3-µm-wide lines in the pattern is approximately 1 nm.

To verify triazole formation on the azido SAM, we compared the reaction between azido SAM **2** and 1-octadecyne induced by µCP and from solution. We used a flat, featureless PDMS stamp to demonstrate triazole formation by µCP. The flat stamp was inked with a solution of 1-octadecyne in ethanol (5 mM) and brought into conformal contact with the azido-terminated SAM for 15 min and with a load of 35 g. After printing, the substrate was rinsed with ethanol and dried with nitrogen. To investigate the reaction with acetylene from solution, the azido-terminated SAM was exposed to a solution of 1-octadecyne in ethanol (5 mM) for 48 h at room temperature. After reaction, the substrate was rinsed with ethanol and dried with nitrogen.

The X-ray photoelectron spectroscopy (XPS) spectrum of bromo-terminated SAM **1** shows a pronounced Br(3d) peak at 70.5 eV (Figure 2). After substitution with NaN_3, the Br(3d) peak disappeared and a N(1s) peak appeared at 400 eV, which confirmed a complete reaction. When 1-octadecyne was introduced over 15 min by µCP using a flat PDMS stamp (**3**) or alternatively in 48 h by reaction from solution (**3***), the intensity of the C(1s) peak increased, while the intensity of the N(1s) peak decreased. The C/N ratio calculated from the elemental composition was 9.7:1 when the triazole monolayer was formed from solution, whereas after printing it was 10.8:1. The theoretical C/N ratio is 10:1. Hence, XPS indicates that the triazole is formed by reaction
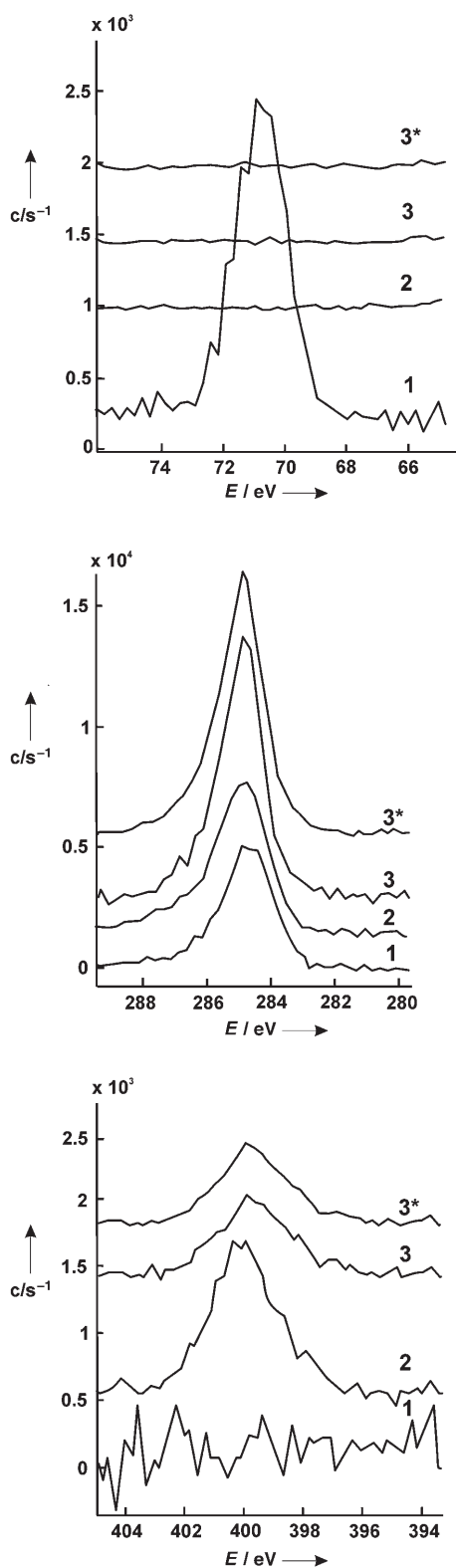
Figure 2. Elemental XPS scans for a) bromine, b) carbon, and c) nitrogen; $E =$ binding energy, $c =$ counts. **1**: Bromo-terminated SAM. **2**: Azido-terminated SAM. **3**: Triazole SAM prepared by μCP of 1-octadecyne on SAM **2** for 15 min. **3***: Triazole SAM prepared by reaction from solution of 1-octadecyne with SAM **2** for 48 h.

from solution as well as by using μCP, but the cycloaddition occurs much faster in the latter case.

Grazing-angle infrared spectroscopy (GAIRS) of bromo-terminated SAM **1** revealed the typical methylene bands at 2924 and 2852 cm^{-1} (Figure 3, **1**). After exposure of the
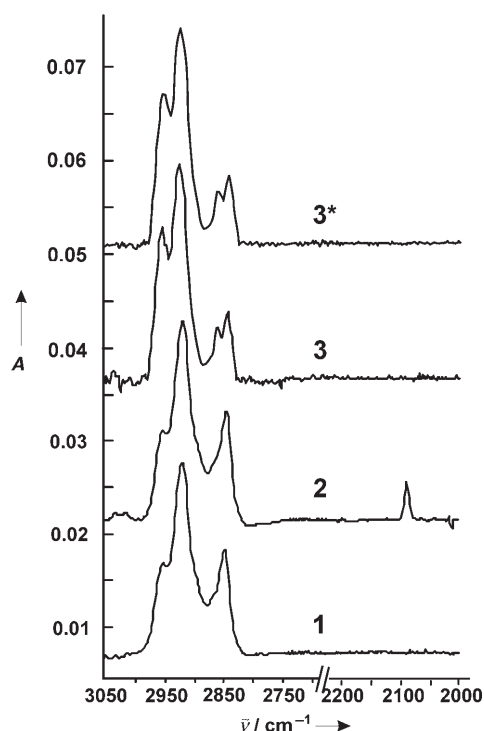

Figure 3. Grazing-angle infrared spectra of functionalized monolayers on a silicon oxide substrate. **1**: Bromo-terminated SAM. **2**: Azido-terminated SAM. **3**: Triazole SAM prepared by μCP of 1-octadecyne on SAM **2** for 15 min. **3***: Triazole SAM prepared by reaction from solution of 1-octadecyne with SAM **2** for 48 h.

bromo-terminated SAM **1** to NaN$_3$, the azido-terminated monolayer showed an absorption at 2089 cm^{-1} ($\nu_{as}(N_3)$) in addition to the methylene absorption bands (Figure 2a, **2**). When SAM **2** was reacted for 48 h with 1-octadecyne from solution (Figure 3, **3***), the azide peak disappeared and in addition two bands responsible for methyl stretching were visible at 2960 ($\nu_{as}(CH_3)$) and 2871 cm^{-1} ($\nu_s(CH_3)$). The bands at 2935 and 2855 cm^{-1} are the methylene stretching modes. After μCP of 1-octadecyne with a flat PDMS stamp for 15 min (Figure 3, **3**), the GAIR spectrum was identical to that obtained for the reaction conducted from solution. In summary, the cycloaddition reaction is confirmed by the disappearance of the $\nu_{as}(N_3)$ peak and the appearance of the $\nu(CH_3)$ peaks.[9,10]

In addition, the cycloaddition reaction of azido SAM **2** with 1-octadecyne was monitored with ellipsometry. It was found that bromo SAM **1** has a thickness $d = 1.34 \pm 0.08$ nm and azido SAM **2** has $d = 1.48 \pm 0.10$ nm, whereas for triazole SAM **3** $d = 2.10 \pm 0.14$ nm and for triazole SAM **3*** $d = 1.88 \pm 0.17$ nm. Hence, the cycloaddition leads to a significant increase in the thickness of the SAM, consistent with the AFM height scan (Figure 1). The average increase in d (0.5–

1.0 nm) indicates that the triazole alkyl chain is substantially tilted relative to the substrate surface.

It should be emphasized that the [2+3] cycloaddition of azides and electron-rich acetylenes such as 1-octadecyne is normally very slow in the absence of a Cu^I catalyst. Indeed, Collman et al.[9a] and Lummerstorfer and Hoffmann[10] reported that no reaction occurs between azido SAMs and electron-rich acetylenes such as 1-octyne and ethynyl ferrocene in the absence of a Cu^I catalyst.[15] To scavenge any adventitious metal-ion contamination in solvents, reactants, PDMS stamps, or substrates, the cycloaddition reaction was carried out in the presence of ethylenediaminetetraacetic acid (EDTA, 0.05 mM; see the Supporting Information). No difference was observed in the reaction of the azido SAM with 1-octadecyne, either by μCP or from solution in the presence of EDTA.

Furthermore, to illustrate the power and scope of the triazole click reaction by μCP, fluorescent alkyne LRA (lissamine rhodamine with a terminal acetylene unit) was printed on azido SAM **2** (Figure 4). The synthesis of LRA is described in the Supporting Information. Prior to printing, the PDMS stamp was oxidized with UV/ozone plasma for 30 min

to increase the wettability and improve the spreading of the polar ink on the stamp. Directly after oxidation the stamp was inked with a solution of LRA in ethanol (1 mM), dried with nitrogen for 1 min, and brought into conformal contact with azido-terminated SAM **2** on a glass slide for 1 min. The substrate was vigorously rinsed with MilliQ water, sonicated in ethanol for 5 min, and dried with nitrogen. It is evident from the confocal microscopy image in Figure 4 that the 20-μm dot features of the stamp are reproduced faithfully on the substrate and that the ink is distributed homogeneously over the contact area.[16] As fluorescent alkyne LRA is still present on the surface in spite of extensive rinsing and sonication, any type of absorption other than covalent immobilization through triazole formation can be excluded. To scavenge any adventitious metal-ion contamination, μCP of LRA on SAM **2** was also performed in the presence of EDTA (0.05 mM). No change was observed (see the Supporting Information). However, when μCP of LRA was performed on an "inert" SAM of *n*-dodecyltriethoxysilane, no evidence of immobilization of LRA was found (see Supporting Information). We explain the remarkable efficiency of the click reaction of LRA printed on the azido SAM by the high local concentration of the polar ink at the surface of the oxidized PDMS stamp.

In conclusion, click chemistry can be applied to the microcontact printing of acetylenes onto azido-terminated SAMs. Synthesis in the nanoscale confinement between a PDMS stamp and a reactive substrate leads to the desired product within a short period of time, without a catalyst, and under mild conditions. We envisage that click chemistry by μCP can be applied using a wide variety of acetylenes and immobilized azides, as well as azides and immobilized acetylenes. In particular, this methodology will be useful for the directed immobilization of (bio)molecules that are modified with either acetylene or azide units. Click chemistry by μCP should serve the development of biological arrays that can be obtained within a short reaction time, under mild reaction conditions with no toxic catalyst required, with high selectivity and quantitative yields, and tolerance for a wide range of functionally complex substances.
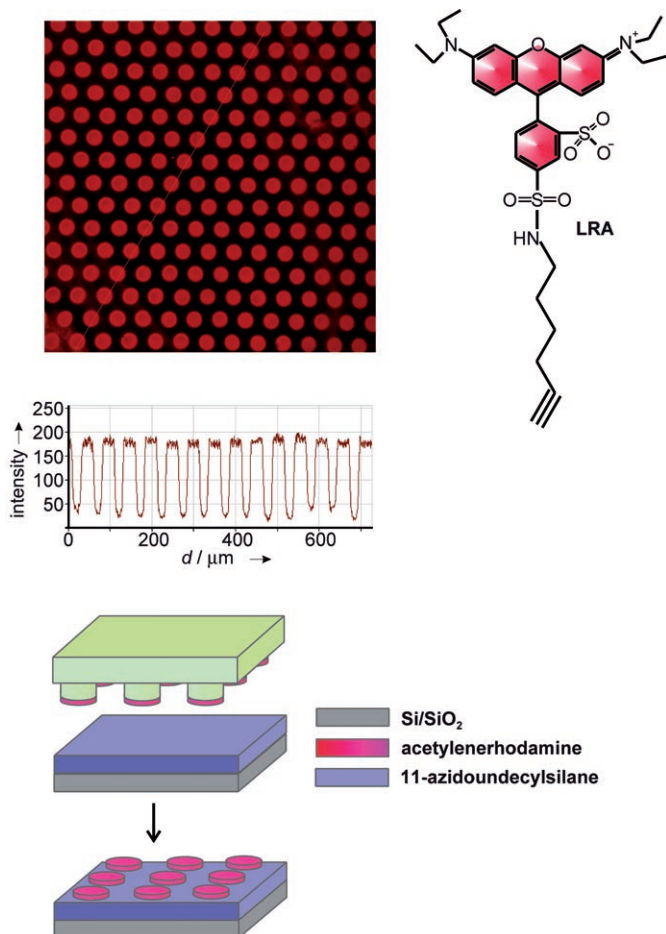
Figure 4. Top: Fluorescence microscopy image (700 × 700 μm) of LRA printed in 20-μm dots onto azido-terminated SAM **2**. Bottom: Schematic representation of the μCP-induced click reaction between the fluorescent alkyne LRA and the azido-terminated silane SAM on a glass slide.

[1] a) A. Kumar, G. M. Whitesides, *Appl. Phys. Lett.* **1993**, *63*, 2002–2004; b) N. L. Jeon, K. Finnie, K. Branshaw, R. G. Nuzzo, *Langmuir* **1997**, *13*, 3382–3391; c) Y. N. Xia, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568–594; *Angew. Chem. Int. Ed.* **1998**, *37*, 551–575; d) B. Michel, A. Bernard, A. Bietsch, E. Delamarche, M. Geissler, D. Juncker, H. Kind, J.-P. Renault, H. Rothuizen, H. Schmid, P. Schmidt-Winkel, R. Stutz, H. Wolf, *IBM J. Res. Dev.* **2001**, *45*, 697–719.

[2] a) L. Yan, X.-M. Zhao, G. M. Whitesides, *J. Am. Chem. Soc.* **1998**, *120*, 6179–6180; b) L. Yan, W. T. S. Huck, X.-M. Zhao, G. M. Whitesides, *Langmuir* **1999**, *15*, 1208–1214; c) J. Lahiri, E. Ostuni, G. M. Whitesides, *Langmuir* **1999**, *15*, 2055–2060.

Communications

[3] T. P. Sullivan, M. L. van Poll, P. Y. W. Dankers, W. T. S. Huck, *Angew. Chem.* **2004**, *116*, 4286–4289; *Angew. Chem. Int. Ed.* **2004**, *43*, 4190–4193.

[4] D. I. Rozkiewicz, B. J. Ravoo, D. N. Reinhoudt, *Langmuir* **2005**, *21*, 6337–6343.

[5] D. I. Rozkiewicz, Y. Kraan, M. W. T. Werten, F. A. de Wolf, V. Subramaniam, B. J. Ravoo, D. N. Reinhoudt, *Chem. Eur. J.*, DOI: 10.1002/chem.200501554.

[6] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[7] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; c) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.

[8] a) T. S. Seo, Z. M. Li, H. Ruparel, J. Y. Ju, *J. Org. Chem.* **2003**, *68*, 609–612; b) A. J. Link, D. A. Tirrell, *J. Am. Chem. Soc.* **2003**, *125*, 11164–11165; c) A. T. Poulin-Kerstien, P. B. Dervan, *J. Am. Chem. Soc.* **2003**, *125*, 15811–15821; d) B. Parrish, R. B. Breitenkamp, T. Emrick, *J. Am. Chem. Soc.* **2005**, *127*, 7404–7410; e) V. P. Mocharla, B. Colasson, L. V. Lee, S. Röper, K. B. Sharpless, C. H. Wong, H. C. Kolb, *Angew. Chem.* **2005**, *117*, 118–122; *Angew. Chem. Int. Ed.* **2005**, *44*, 116–120; f) M. Whiting, J. Muldoon, Y.-C. Lin, S. M. Silverman, W. Lindstrom, A. J. Olson, H. C. Kolb, M. G. Finn, K. B. Sharpless, J. H. Elder, V. V. Fokin, *Angew. Chem.* **2006**, *118*, 1463–1467; *Angew. Chem. Int. Ed.* **2006**, *45*, 1435–1439.

[9] a) J. P. Collman, N. K. Devaraj, C. E. D. Chidsey, *Langmuir* **2004**, *20*, 1051–1053; b) J. K. Lee, Y. S. Chi, I. S. Choi, *Langmuir* **2004**, *20*, 3844–3847; c) R. Zirbs, F. Kienberger, P. Hinterdorfer, W. H. Binder, *Langmuir* **2005**, *21*, 8414–8421; d) N. K. Devaraj, P. H. Dinolfo, C. E. D. Chidsey, J. P. Collman, *J. Am. Chem. Soc.* **2006**, *128*, 1794–1795.

[10] T. Lummerstorfer, H. Hoffmann, *J. Phys. Chem. B* **2004**, *108*, 3963–3966.

[11] H. Li, F. Cheng, A. M. Duft, A. Adronov, *J. Am. Chem. Soc.* **2005**, *127*, 14518–14524.

[12] D. D. Diaz, S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin, M. G. Finn, *J. Polym. Sci. A* **2004**, *42*, 4392–4403.

[13] N. K. Devaraj, G. P. Miller, W. Ebina, B. Kakaradov, J. P. Collman, E. T. Kool, C. E. D. Chidsey, *J. Am. Chem. Soc.* **2005**, *127*, 8600–8601.

[14] N. Balachander, C. Sukenik, *Langmuir* **1990**, *6*, 1621–1627.

[15] Substitution and addition reactions on SAMs are notoriously sensitive to steric constraints, surface coverage of functional groups, reactant transport limitations, solvation effects, charge, and dipole effects: a) D. G. Kurth, T. Bein, *Langmuir* **1993**, *9*, 2965–2973; b) T. P. Sullivan, W. T. S. Huck, *Eur. J. Org. Chem.* **2003**, 17–29.

[16] The intensity profile in Figure 4 demonstrates that LRA spreads to the noncontacted area of the substrate. Overinking and ink spreading are common phenomena in μCP.