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Heterogeneous Diazo-Transfer Reaction: A Facile Unmasking of Azide Groups on Amine-Functionalized Insoluble Supports for Solid-Phase Synthesis

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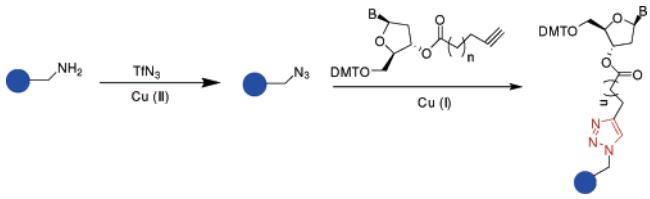

Solid-supported azides are commonly generated through direct nucleophilic displacement of appropriately activated supports by the azide ion. This reaction usually proceeds rather sluggishly under harsh conditions. Here, we report that triflyl azide rapidly reacts with a series of amine-functionalized solid supports to generate azide-coated supports under mild conditions. Further, we demonstrate that the "azide coat" allows facile loading of alkyne-functionalized leader nucleoside monomers by click chemistry. Finally, we show that the nucleoside-functionalized supports are suitable for solid-phase oligonucleotide synthetic applications. The approach herein described extends the scope of the amine−azide conversion reaction and may be adaptable for the introduction of azide to diverse amine-terminated solid supports that are not easily accessible by the conventional nucleophilic displacement method.

Introduction

Solid-phase synthesis (SPS) has become a routine and unmatched method for the synthesis of biomacromolecules such as peptide, DNA, and RNA.[1] Vital to the success of SPS is an optimal covalent attachment of the leader monomer onto insoluble supports. A majority of the protocols for the immobilization of the leader monomer onto solid supports are based on alkylation and acylation reactions. However, these protocols usually require special procedures which, in untrained hands, are often difficult and time consuming. For example, attachment of a substrate nucleoside onto a commercially available insoluble solid support such as 3-aminopropylated-CPG could take days to accomplish, under rigorous exclusion of moisture.[2] Also, partial support loading could result in unwanted side reactions which could compromise product quality. To address these problems and expand the scope of functionalities which can serve as a handle for SPS, a large collection of linkers have been investigated and developed.[3−6] However, the vast majority of these linkers still relies on the traditional coupling protocols for the monomer support attachment. It is therefore of prime interest to have flexible synthetic methodologies for rapid loading of leader monomers onto the supports. This realization has continued to spawn immense efforts in the literature.[7−12]

(1) (a) Merrifield, B. *Protein Sci.* **1997**, *5*, 1947. (b) Caruthers, M. H. *Science* **1985**, *230*, 281. (c) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223.

(2) Pon, R. T. Attachments of Nucleosides to Solid Supports. *In Current Protocols in Nucleic Acids Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley: New York, 1999; pp 287−298.

(3) Hall, S. E. *Mol. Diversity* **1999**, *4*, 131.

(4) Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091.

(5) Wills, A. J.; Balasubramanian, S. *Curr. Opin. Chem. Biol.* **2003**, *7*, 346.

(6) Dolle, R. E. *J. Comb. Chem.* **2003**, *5*, 693.

(7) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165.

(8) MacMillan, A. M.; Verdine, G. L. *J. Org. Chem.* **1990**, *55*, 5931.

(9) Nilsson, B. L.; Soellner, M. B.; Raines, R. T. *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 91.

(10) Canne, L. E.; Botti, P.; Simon, R. J.; Chen, Y.; Dennis, E. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1999**, *121*, 8720.

(11) Stetsenko, D. A.; Malakhov, A. D.; Gait, M. J. *Org. Lett.* **2002**, *4*, 3259.

(12) Kahl, J. D.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 597−604.

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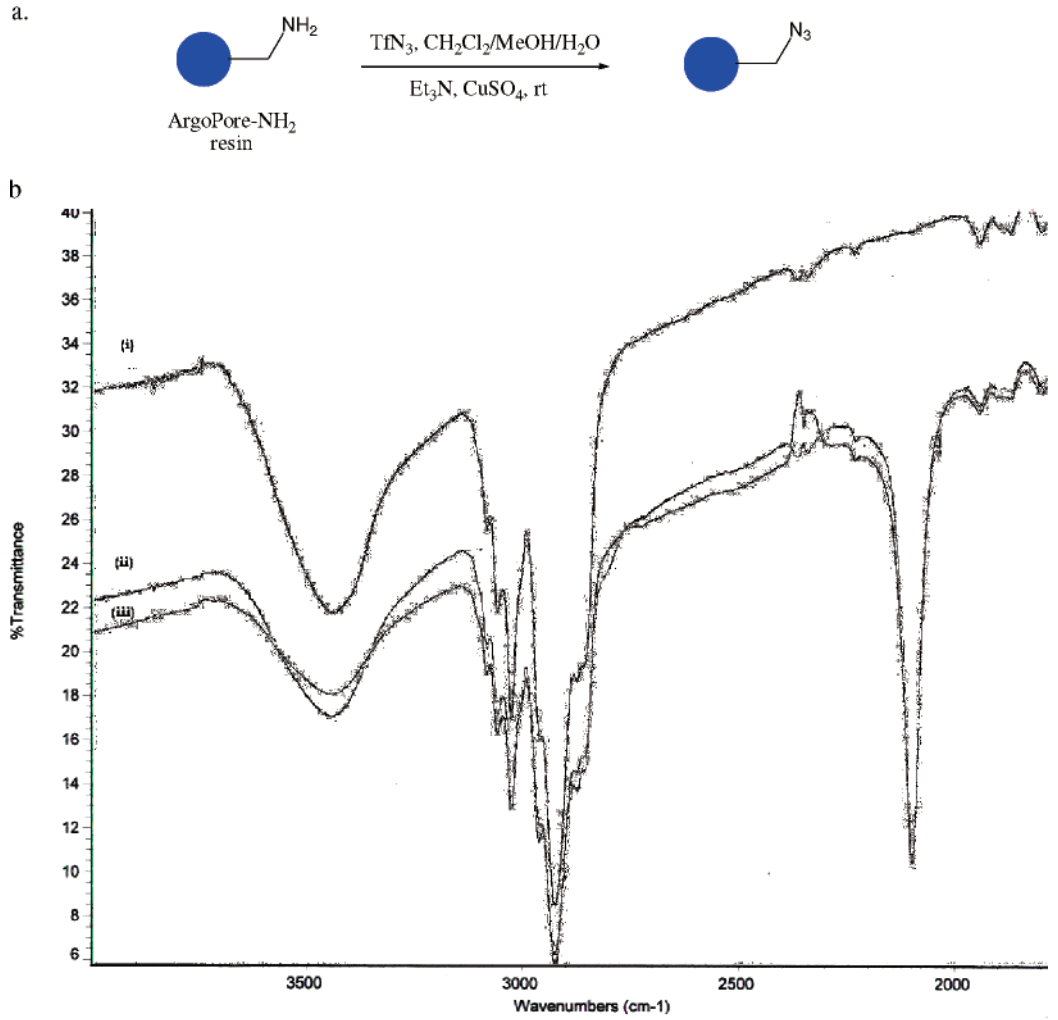


FIGURE 1. Analysis of heterogeneous Diazo-transfer reactions. (a) Conditions for heterogeneous diazo-transfer reaction (the blue circle represents the part of the resin that is unaffected by the reaction). (b) FTIR spectra (KBr) of Cu(II)-catalyzed reaction: (i) unmodified resin, (ii) 20 min reaction time, and (iii) 21 h reaction time.

The utility of organic azides in bioconjugations and synthetic organic chemistry applications is enjoying a renaissance. They are extensively used in photoaffinity labeling of biomacromolecules,[13] the Staudinger ligation reaction,[14] and a protection strategy for amines.[15] Moreover, with the recent discovery of Cu(I) catalysis, termed click chemistry by Sharpless and coworkers,[16] Huigsen cycloaddition reaction between azides and terminal alkynes has become the premier conjugation technique in chemistry, biology, and material science applications. Click chemistry has allowed rapid construction of complex macromolecules, whole cell and organism modification, and small molecules with diverse biological properties.[17]

There are several methods for the synthesis of organic azides.[18,19] Among these are substitution reactions between various electrophiles and azide nucleophiles to generate aliphatic azides. Aromatic azides can be easily prepared by diazotization of the corresponding amines[18,20] and reaction of aryl Grignard or lithium reagents and aryl amide salts with *para*-tosylazide.[21,22] Solid-supported azides have been principally generated by direct

(13) For reviews on aryl azides as photoaffinity labels and references therein, see: (a) Bayley, H.; Staros, J. V. In *Azides and Nitrenes*; Scriven, E. F. V., Ed.; Academic Press: Orlando, 1984; pp 433−490. (b) Applications of Photochemistry in Probing Biological Targets. In *Annals of the New York Academy of Sciences*; Tometsko, A. M., Richards, F. M., Eds.; New York Academy of Sciences: New York, 1980; Vol. 346. (c) Radominska, A.; Drake, R. R. *Methods Enzymol.* **1994**, *230*, 330. (d) Fedan, J. S.; Hogaboom, G. K.; O'Donnell, J. P. *Biochem. Pharmacol.* **1984**, *33*, 1167.

(14) (a) Dube, D. H.; Bertozzi, C. R. *Curr. Opin. Chem. Biol.* **2003**, *7*, 616. (b) Vocadlo, D. J.; Hang, H. C.; Kim, E. J.; Hanover, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9116. (c) Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873.

(15) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773.

(16) Numerous examples of click chemistry have appeared in the literature. Cited here are two pioneering examples: (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

(17) For reviews on the application of click chemistry, see: (a) Kolb, H. C.; Sharpless, K. B. *DDT* **2003**, *8*, 1128. (b) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51.

(18) Sandler, S.; Karo, W. *Organic Functional Group Preparations*; Academic Press: New York, 1971; pp 268−284.

(19) Scriven, E. F. V.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297.

(20) For a review, see: (a) Biffin, M. E. C.; Miller, J.; Paul, D. B. In *The Chemistry of the Azido Group*; Patai, S., Ed.; Wiley: New York, 1971; pp 147−176. See also: (b) Kauer, J. C.; Carboni, R. A. *J. Am. Chem. Soc.* **1967**, *89*, 2633. (c) Takahashi, M.; Suga, D. *Synthesis* **1998**, *7*, 986.

TABLE 1. Quantification of the Extent of Heterogeneous Diazo-Transfer Reactions of ArgoPore-NH_2 Resin in the Presence (a) and Absence (b) of Cu(II) Catalyst (Quantitative Ninhydrin Analysis)

(a)				(b)			
reaction time (min)	mmol/g of NH_2	mmol/g of N_3[a]	% NH_2 conversion	reaction time (min)	mmol/g of NH_2	mmol/g of N_3[a]	% NH_2 conversion
0	1.174[b]			0	1.174		
20	0.126	1.048	89.3	20	0.683	0.491	41.8
60	0.084	1.090	92.8	60	0.260	0.914	77.9
240	0.048	1.126	95.9	240	0.142	1.032	87.9
1260	0.057	1.117	95.1[c]	1260	0.039	1.135	96.7

[a] mmol/g of N_3 was calculated from the difference between mmol/g of NH_2 of unmodified resin and mmol/g of NH_2 at a given time during the course of the reaction. [b] Standard loading of ArgoPore-NH_2 resin; the recommended manufacturer loading capacity is 0.90 mmol/g. [c] Within the limit of assay error obtained from the standard deviation from two experiments.

TABLE 2. Heterogeneous Diazo-Transfer Reaction Explored on Amine-Terminated Resins

type of resins	reaction temp (°C)	azide conversion[a] (%)	reaction time (h)
ArgoPore	25	90	4
polystyrene A	25	90	4
(aminoethyl) polystyrene	25	90	4
tentagel	25	90	4
CPG	37	50	24

[a] Calculated from quantitative ninhydrin analysis.

nucleophilic displacement of appropriately activated supports.[23−25] This reaction usually proceeds rather sluggishly under harsh conditions. More conveniently, a direct conversion of organic amines to azides (diazo-transfer) is commonly achieved by the reaction of the corresponding amine with triflyl azide [***Caution!* Triflyl azide may be explosive, and it should be handled with care. However, we have not experienced any difficulties in handling triflyl azide**].[15,26] Diazo transfer is a high-yielding reaction that proceeds under mild conditions, and it is especially useful for the synthesis of tertiary azides where steric hindrance precludes the traditional synthesis through a direct nucleophilic displacement of leaving groups such as halides and sulfates. Numerous amine-attached insoluble solid supports, whose surfaces offer a challenging steric maze that may be well-suited for diazo-transfer reaction, are commercially available. Inspired by this possibility, we investigated the feasibility of a heterogeneous diazo-transfer reaction. We report in this paper that triflyl azide rapidly reacts with a series of amine-functionalized solid supports to generate azide-coated supports. The "azide coat" was further shown to allow a facile loading of alkyne-functionalized leader nucleoside monomers and subsequent assembly of oligonucleotides by conventional SPS methods.

These results extend the scope of the amine−azide conversion reaction and also demonstrate the suitability of click chemistry in the support attachment of leader nucleoside monomers for solid-phase oligonucleotide synthetic applications.

Results and Discussion

Heterogeneous Diazo-Transfer Reaction. First, we investigated the possibility of reaction of triflyl azide with ArgoPore-NH_2 resin. The diazo-transfer reaction was initiated with a 7 mol excess of freshly prepared triflyl azide (Figure 1a).[26e,h] The reaction progress was monitored by quantitative ninhydrin analysis and FTIR. We noticed a clear distinction between solution-phase and heterogeneous diazo-transfer reaction; namely, the heterogeneous reaction proceeded rapidly in the presence of Cu(II) catalyst.[26e] The reaction reached its maximum within 4 h as judged by quantitative ninhydrin analysis[27] (Table 1), and the presence of an azide moiety was confirmed by FTIR (2097 cm^{-1})[28] (Figure 1b). In the absence of Cu(II) catalyst, the reaction proceeded at a much slower rate, reaching maximal conversion at 21 h (Table 1).

On the basis of this interesting result, we sought to probe the generality of this reaction. We turned to investigate the reactivity of four additional commercial amine-terminated resins, namely, aminopropylated-CPG, Tentagel S-NH_2, polystyrene A-NH_2, and (aminomethyl) polystyrene. These resins each offer different arrays of polymeric backbones and conceivably varied steric environments. Tentagel S-NH_2, polystyrene A-NH_2, and (aminomethyl) polystyrene reacted with triflyl azide in a fashion similar to that for the ArgoPore resin yielding azide-coated resins in excellent yields (Table 2). However, aminopropylated-CPG failed to react with triflyl azide under conditions identical to those used for the ArgoPore resin. We then tested various solvents and temperature conditions and found that the reaction occurred at slightly elevated temperature, between 37 and 40 °C, to give azide-coated aminopropylated-CPG in 50% yield within 24 h (Table 2). The introduction of azide onto solid supports by the conventional nucleophilic displacement reactions required the synthesis of activated supports or the use of limited

(21) See, for example: (a) Smith, P. A. S.; Rowe, C. D.; Bruner, L. B. *J. Org. Chem.* **1969**, *34*, 3430. (b) Smith, P. A. S.; Budde, G. F.; Chou, S.-S. P. *J. Org. Chem.* **1985**, *50*, 2062. (c) Gavenonis, J.; Tilley, T. D. *J. Am. Chem. Soc.* **2002**, *124*, 8536. (d) Gavenonis, J.; Tilley, T. D. *Organometallics* **2002**, *21*, 5549.

(22) (a) Fisher, W.; Anselme, J.-P. *J. Am. Chem. Soc.* **1967**, *89*, 5284. (b) Nielsen, P. E. *Tetrahedron Lett.* **1979**, *20*, 2705.

(23) Arseniyadis, S.; Wagner, A.; Mioskowski, C. *Tetrahedron Lett.* **2002**, *43*, 9717.

(24) Lober, S.; Rodriguez-Loaiza, P.; Gmeiner, P. *Org. Lett.* **2003**, *5*, 1753.

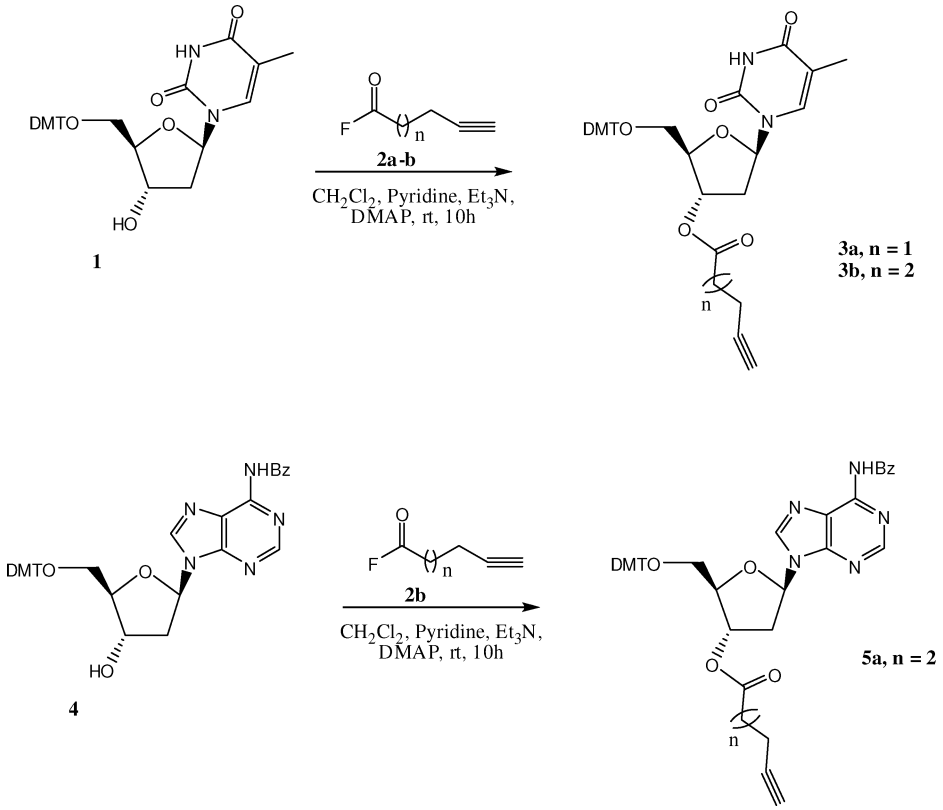
(25) Lober, S.; Gmeiner, P. *Tetrahedron* **2004**, *60*, 8699.

(26) For examples, see: (a) Ruff, J. K. *Inorg. Chem.* **1965**, *4*, 567. (b) Caveander, C. J.; Shiner, V. J. *J. Org. Chem.* **1972**, *37*, 3567. (c) Zaloom, J. R.; David, C. *J. Org. Chem.* **1981**, *46*, 5173. (d) Eaton, P. E.; Fisher, A. M.; Hormann, R. E. *Synlett* **1990**, 737. (e) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029. (f) Ding, Y.; Swayze, E. E.; Hofstadler, S. A.; Griffey, R. H. *Tetrahedron Lett.* **2000**, *41*, 4049. (g) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527. (h) Liu, Q.; Tor, Y. *Org. Lett.* **2003**, *5*, 2571. (i) Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. *Tetrahedron Lett.* **2006**, *47*, 2383−2385. (j) We are grateful to the reviewers for suggesting the substitution of dichloromethane with nonhalogenated solvents as an alternative way to minimize the potential danger of explosion during the synthesis of triflyl azide.

(27) Sarin, V. K.; Kent, S. B.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.

(28) The N_3 group asymmetric vibration for most organic azides is in the range of 2170−2080 cm^{-1}. Lieber, E.; Ramachandra Rao, C. N.; Chao, T. S.; Hoffman, C. W. W. *Anal. Chem.* **1957**, *29*, 916.

SCHEME 1. Synthesis of Alkyne-Functionalized Nucleosides



commercial activated supports such as Merrifield resin.[23−25] These reactions proceed slowly under extreme conditions. Operationally, however, the current heterogeneous diazo-transfer reaction proceeds rapidly and smoothly in a conventional reaction flask at ambient conditions without any need for elaborate technical handling.

Synthesis of Alkyne-Functionalized Nucleosides. Among the bioconjugation protocols utilizing organic azides, Cu(I)-catalyzed alkyne−azide cycloaddition reaction (Sharpless click chemistry)[16] has attracted the most attention in macromolecular fabrication. The application of click chemistry in the immobilization of oligonucleotide probes on self-assembled monolayers on thiol-coated silicon wafers has recently appeared in the literature.[29] Similarly, click chemistry has been used to directly functionalize resins for the solid-phase synthesis of a library of dopaminergic arylcarbamides,[24] tertiary amines,[25] and peptidotriazole.[16b,30] As an extension of the application of this versatile chemistry, we explored the suitability of two of the synthesized azido resins in the loading of nucleoside leader monomers for solid-phase oligonucleotide synthesis. Toward this end, we have prepared new alkyne-functionalized nucleosides **3a**, **3b**, and **5a**. Acylation of 5′-*O*-dimethoxytritylthymidine **1** with acid fluorides **2a**,**b** adapting the procedure described by Oliver and Oyelere furnished 3′-alkyne esters **3a** and **3b** in good yields.[31] Similarly, 2′-deoxyadenosine-3′-alkyne ester **5a** was obtained from acylation of commercially available protected 2′-deoxyadenosine **4** and acid fluoride **2b** (Scheme 1). These alkyne-functionalized nucleosides were evaluated as substrates for attachment to azide-coated supports by click chemistry.

Attachment of Leader Alkyne Monomers to Solid Supports and Solid-Phase Oligonucleotide Synthesis. The azide-coated resins were first treated with acetic anhydride to cap any traces of unreacted amines using a standard protocol. To identify the optimum reaction conditions for the attachment of the alkyne nucleosides onto azide-coated supports, we tested resin derivatization of alkynes **3a** and **3b** by copper-catalyzed Huigsen reaction on ArgoPore-N_3 resin (Figure 2).

In a typical reaction, **3a** or **3b** was incubated with ArgoPore-N_3 resin and Cu(I) in THF and a Hunig's base mixture in the presence of tris(benzyltriazolylmethyl)-amine (TBTA).[32] The reaction proceeded smoothly at ambient conditions resulting in an uneventful resin loading. Quantitative trityl group[33] analysis revealed that as little as 0.5 equiv of the alkyne **3a** or **3b** is sufficient for a near quantitative nucleoside resin loading within 2 h. Because such a maximal resin loading may not be ideal for an SPS application, a series of loading reactions were then investigated to obtain nucleoside resin loadings at typical concentrations for solid-phase oligonucleotide synthesis. We found that the reaction of about 0.1 equiv of **3a** or **3b** resulted in ArgoPore-N_3 resin loading of about 7−10 μmol/g within 30 min, and a prolonged reaction time resulted in increased nucleoside attachment. For example, within 2 h, a loading of about 30−46 μmol/g was obtained. We saw no significant difference in the loading capacity of compounds **3a** and **3b**. Interestingly, about 10 times as much compound **5a** is necessary

(29) Devaraj, N. K.; Miller, G. P.; Ebina, W.; Kakaradov, B.; Collman, J. P.; Kool, E. T.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **2005**, *127*, 8600.

(30) (a) Tornoe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J Comb. Chem.* **2004**, *6*, 312. (b) Zhang, Z.; Fan, E. *Tetrahedron Lett.* **2006**, *47*, 665.

(31) Oliver, J. S.; Oyelere, A. *J. Org. Chem.* **1996**, *61*, 4168.

(32) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853.

(33) Applied Biosystems Users Bulletin. DNA Synthesizer, Model 380/381, Issue 13, Revised April 1, 1987; p 12.

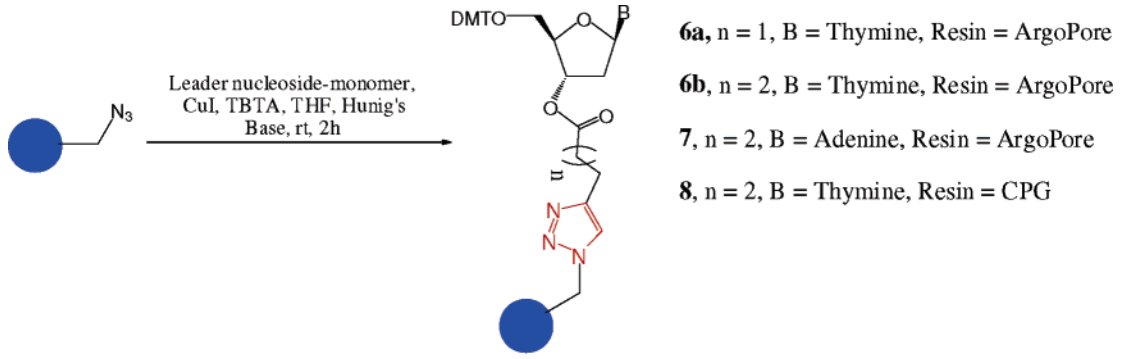


FIGURE 2. Azido resin derivatization with an alkyne monomer by Cu(I)-catalyzed Huigsen reaction.

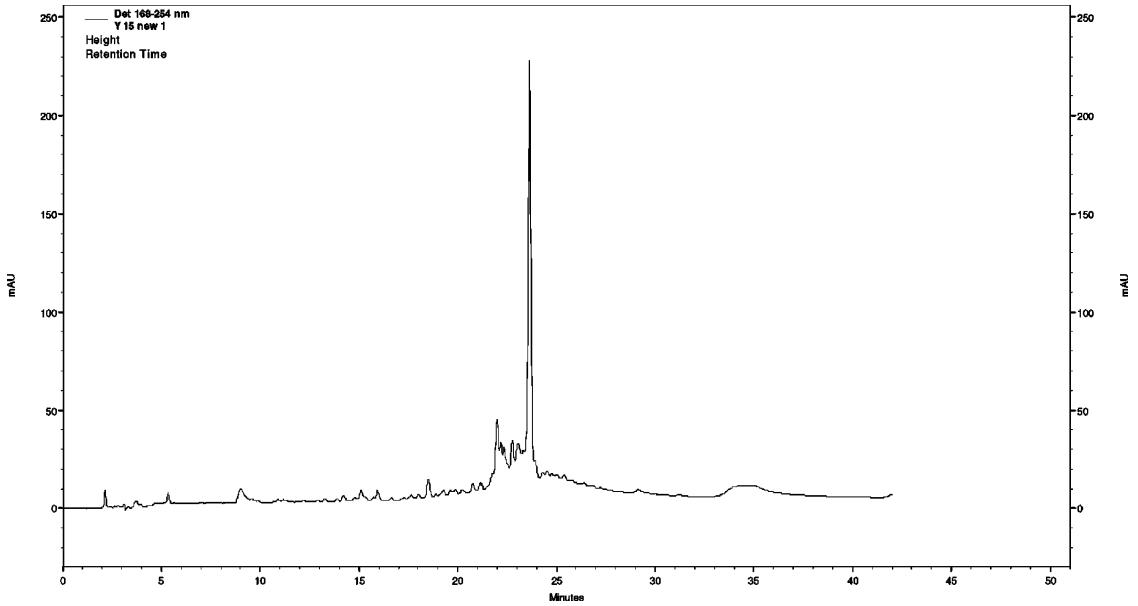


FIGURE 3. Reverse-phase HPLC elution pattern of a crude oligonucleotide. Deprotected crude 15mer (AGC CAG ATT TGA GCT). HPLC conditions: Phenomenex RP C-18 column; solvent A, 0.1 M triethylammonium acetate; solvent B, acetonitrile; gradient of 6−28% of solvent B in 45 min; flow rate of 1 mL/min.

to obtain nucleoside loadings comparable to that of **3a** or **3b**; we could not, however, ascertain the source of this coupling disparity. Because of the wide application of aminopropylated-CPG resin in solid-phase oligonucleotide synthesis, we turned our attention to study the coupling behavior of CPG-N_3 resin. We found that using 2 mol equiv of compound **3b** under conditions similar to those described for ArgoPore-N_3 resin resulted in coupling yields of 40 ± 5 μmol/g within 2 h (Figure 2).

Encouraged by these results, we then decided to investigate the suitability of this solid-support coupling protocol in solid-phase oligonucleotide synthesis. Thymidine- and deoxyadenosine-linked ArgoPore resins **6** (**a** or **b**) and **7**, respectively, and thymidine-linked CPG resin **8** prepared above were employed in automated oligonucleotide synthesis in Expedite[34] and Applied Biosystems DNA synthesizers using phosphoramidite chemistry.[1c,35] In these experiments, we synthesized on a 1 μmol scale a 15mer (AGC CAG ATT TGA GCT) and a 30mer (AGC CAG ATT TGA GCT TGG GGC TCT CTG GCT) on thymidine-immobilized supports and a 30mer (TGC CAG ATT TGA GCT TGG GGC TCT CTG GCA) on a 2′-deoxyadenosine-immobilized support. Oligonucleotide syntheses and the subsequent deprotection and cleavage of the support-bound products followed standard procedures.[35] Figure 3 shows the reversed-phase HPLC elution pattern of a representative crude product. This HPLC profile compared favorably well with that of similar oligo made from commercial resins. The identities of the HPLC-purified oligos were further confirmed by UV−vis absorption and MALDI-TOF MS analysis (see Supporting Information for details).

In summary, we have demonstrated the feasibility of heterogeneous diazo-transfer reaction on commercial, amine-terminated resins. We also showed that the so-formed azide coat can be used to load leader nucleoside monomers on solid supports using click chemistry under benign experimental conditions. This approach avoids expensive, toxic, and moisture-sensitive additives that are required for traditional monomer support attachment by esterification reactions. Furthermore, we demonstrated the potential utility of the support coupling protocol, herein described, in oligonucleotide solid-phase synthetic ap-

(34) DNA syntheses on an Expedite DNA synthesizer were performed at the Yale University Keck Oligo facility.

(35) Agrawal, S. *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogues*; Humana Press: NJ, 1993; Vol. 20.

plications. Part of our ongoing work is synthesis of second-generation linkers that will allow facile cleavage of SPS assembled macromolecules from various insoluble supports.

Experimental Section

Tentagel S-NH_2, polystyrene A-NH_2, and (aminomethyl) polystyrene, ArgoPore-NH_2 and aminopropylated-CPG resins were obtained from commercial suppliers. Cyanuric fluoride was obtained and used without further purification. Anhydrous solvents and other reagents were purchased and used without purification. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and the spots were examined with UV light. Column chromatography was carried out on 200−400 mesh silica gel. Triflyl azide was prepared as described by Titz et al. and Liu and Tor.[26h,i] Acid fluorides were made according to Oliver and Oyelere,[31] and 5′-*O*-dimethoxytritylthymidine **1** was prepared following the procedure of Meier et al.[36] Oligonucleotide HPLC purification was performed using a Phenomenex RP C-18 column, eluting with a gradient of 6−28% acetonitrile in 0.1 M triethylammonium acetate (pH = 7.1).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-pent-4-ynoatethymidine (3a). 5′-*O*-Dimethoxytritylthymidine (**1**) (0.750 g, 1.38 mmol) was added to an oven-dried round-bottom flask (10 mL) under argon and coevaporated with anhydrous pyridine (2 × 5 mL). Dry dichloromethane (4 mL), dry pyridine (0.4 mL), and dry triethylamine (0.5 mL) were added under argon. A solution of pent-4-ynoyl fluoride (**2a**) (0.200 g, 2.00 mmol) in dry dichloromethane (1 mL) was added to the solution, and the mixture was stirred at room temperature under argon for 24 h. The reaction was partitioned between dichloromethane (40 mL) and saturated sodium bicarbonate (30 mL). The two layers were separated, and the organic layer was washed with saturated sodium bicarbonate (30 mL) and saturated brine (30 mL) and dried over Na_2SO_4. Solvent was concentrated, and the crude material was purified by flash chromatography (silica gel, step gradient 8:1; 6:1 CH_2Cl_2/acetone) to give 0.560 g (64%) of **3a**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (3H, s), 2.01 (1H, t, J = 2.4 Hz), 2.51 (6H, m), 3.46 (2H, m), 3.77 (6H, s), 4.13 (1H, br s), 5.47 (1H, d, J = 5.6 Hz), 6.44 (1H, m), 6.82 (4H, d, J = 8.8 Hz), 7.22−7.38 (9H, m), 7.59 (1H, s), 9.10 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 14.3, 33.1, 37.9, 55.2, 63.6, 69.6, 75.8, 81.9, 83.9, 84.2, 87.1, 111.7, 113.2, 127.2, 128.0, 128.1, 130.0, 130.3, 135.0, 135.1, 135.3, 144.1, 150.6, 158.7, 158.7, 163.8, 171.3. HRMS (FAB, thioglycerol) calcd for $[C_{36}H_{36}N_2O_8 + H]^+$ 625.2550, found 625.2544.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-hex-5-ynoatethymidine (3b). Reaction of 5′-*O*-dimethoxytritylthymidine (**1**) (0.750 g, 1.38 mmol) and hex-5-ynoyl fluoride (**2b**) (0.229 g, 2.00 mmol) for 24 h as described for compound **3a** followed by flash chromatography (silica gel, step gradient 8:1; 6:1 CH_2Cl_2/acetone) gave 0.571 g (65%) of chromatographically pure **3b**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (3H, s), 1.82 (2H, m), 1.97 (1H, t, J = 2.6 Hz), 2.25 (2H, m), 2.46 (4H, m), 3.45 (2H, m), 3.77 (6H, s), 4.11 (1H, br. s), 5.44 (1H, d, J = 4.8 Hz), 6.43 (1H, app. t), 6.82 (4H, d, J = 8.8 Hz), 7.22−7.38 (9H, m), 7.59 (1H, s), 9.20 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 17.7, 23.2, 32.7, 37.9, 55.2, 63.6, 69.5, 75.3, 82.9, 83.9, 84.2, 87.1, 111.7, 113.2, 127.1, 128.0, 128.0, 130.0, 130.0, 135.0, 135.1, 135.3, 144.1, 150.6, 158.7, 158.7, 163.8, 172.5. HRMS (FAB, thioglycerol) calcd for $[C_{37}H_{38}N_2O_8 + H]^+$ 638.2628, found 638.2606.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(benzoyl)-2′-deoxy-3′-*O*-hex-5-ynoateadenosine (5a). Reaction of protected nucleoside (**4**) (0.200 g, 0.30 mmol), hex-5-ynoyl fluoride (**2b**) (0.114 g, 1.00 mmol), and a catalytic amount of DMAP in dichloromethane (4 mL), dry pyridine (0.4 mL), and dry triethylamine (0.5 mL) for 2 h followed by flash chromatography (silica gel, step gradient 12:1; 10:1 CH_2Cl_2/acetone) gave 0.184 g (82%) of chromatographically pure **5a**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.82 (2H, m), 1.99 (1H, t, J = 2.6 Hz), 2.25 (2H, m), 2.50 (2H, app. t), 2.66 (1H, m), 2.98 (1H, m), 3.43 (2H, d, J = 4.0 Hz), 3.74 (6H, s), 4.28 (1H, m), 5.53 (1H, d, J = 6.0 Hz), 6.50 (1H, m), 6.77 (4H, d, J = 8.8 Hz), 7.17−7.29 (9H, m), 7.36 (2H, d, J = 7.2 Hz), 7.47 (2H, app. t), 7.56 (1H, app. t), 8.00 (2H, d, J = 8.4 Hz), 8.17 (1H, s), 8.69 (1H, s), 9.14 (1H, br. s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.7, 23.3, 32.7, 38.2, 55.2, 63.5, 69.5, 75.2, 82.9, 84.4, 84.5, 86.7, 113.1, 127.0, 127.8, 127.9, 128.0, 128.8, 129.9, 130.0, 132.7, 133.5, 135.4, 144.1, 144.3, 149.3, 151.6, 152.6, 158.5, 164.7, 172.3. HRMS (FAB, thioglycerol) calcd for $[C_{44}H_{41}N_5O_7 + H]^+$ 752.3084, found 752.3110.

Representative Procedure for Heterogeneous Diazo-Transfer Reaction. ArgoPore-N_3 Resin. ArgoPore-NH_2 (1.0 g, 0.90 mmol/g) was swelled in a homogeneous mixture of CH_2Cl_2 (5.6 mL), MeOH (5.2 mL), distilled H_2O (4.9 mL), and Et_3N (0.21 mL) for 2 h. After 2 h, the swelling solution was decanted. Triflyl azide (7.0 mmol) in CH_2Cl_2 (12 mL) and $CuSO_4$ (0.011 g, 0.04 mmol) in distilled H_2O (0.13 mL) were added to the swelled resin and gently stirred (or gently rocked) at 25 °C. Small portions of the resin were taken out at 10 min, 20 min, and 40 min 1 h, 2 h, 4 h, and 21 h for quantitative ninhydrin analysis. The resin was washed in succession with swelling solvent, conc. NH_4OH (2 × 10 mL), and swelling solvent and dried in vacuo to give a brownish solid. IR 2097 cm^{-1}. [Note: the reaction worked equally well when CH_2Cl_2 was replaced with toluene. Because of its better safety profile,[26i] we recommend toluene as a solvent of choice.]

Polystyrene A-N_3 Resin. This resin was synthesized from polystyrene A-NH_2 as described for the synthesis of ArgoPore-N_3 from ArgoPore-NH_2 to give a brown−green solid. IR 2090 cm^{-1}.

(Aminomethyl) Polystyrene-N_3 Resin. This resin was synthesized from (aminomethyl) polystyrene as described for the synthesis of ArgoPore-N_3 from ArgoPore-NH_2 to give a light yellow solid. IR 2092 cm^{-1}.

Tentagel-N_3 Resin. This resin was synthesized from Tentagel S-NH_2 as described for the synthesis of ArgoPore-N_3 from ArgoPore-NH_2 to give a greenish solid. IR 2106 cm^{-1}.

CPG-N_3 Resin. This resin was synthesized from CPG-NH_2 as described for the synthesis of ArgoPore-N_3 from ArgoPore-NH_2 except that the reaction was stirred at 37 °C for 24 h to give a white−blue solid. IR 2359, 2341 cm^{-1}.

Representative Protocol for Nucleoside Loading. In a typical reaction, a mixture of **3a** (0.019 g, 0.03 mmol), ArgoPore-N_3 resin (0.065 g, approx 0.06 mmol), Cu(I) (0.003 g, 0.02 mmol), and TBTA (0.009 g, 0.02 mmol) in THF (1.2 mL) and Hunig's base (12 μL) was shaken at room temperature for 2 h. The resin was filtered off, washed with THF and CH_2Cl_2, and dried in vacuo. Quantitative DMT group[33] analysis gave a nucleoside loading of approximately 0.5 mmol/g.

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Supporting Information Available: UV absorbance spectra after quantitative ninhydrin assay, HPLC traces, and MALDI-TOF MS analyses of purified oligonucleotides and proton and carbon NMR spectra for all compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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(36) Meier, C.; Neumann, J.-M.; André, F.; Henin, Y.; Dinh, T. H. *J. Org. Chem.* **1992**, *57*, 7300.