(4), 126 (4), 125 (1), 124 (1), 122 (0.9), 121 (0.8), 114 (0.8), 113 (7), 112 (4), 111 (2), 110 (1), 99 (10), 98 (6), 97 (7), 96 (2), 88 (5), 86 (30), 85 (33), 84 (53), 83 (11), 82 (3), 81 (2), 71 (55), 70 (11), 69 (11), 68 (3), 67 (2), 58 (6), 57 (100), 56 (22), 55 (21); exact mass calcd 794.425, found 794.418.

For **65**: TLC (silica gel) R_f 0.18 (ethyl acetate), 0.17 (5% methanol in dichloromethane); UV (ethanol) λ_{max} 216, 222, 262, 289, 295, 305 nm; IR (KBr) ν_{max} 3441, 3023, 2959, 2952, 2931, 2895, 2877, 2849, 2843, 2816, 1741, 1672, 1617, 1597, 1500, 1461, 1433, 1371, 1335, 1299, 1247, 1234, 1227, 1198, 1169, 1144, 1132, 1120, 1111, 1087, 1040, 1010, 738 cm^{-1}; 250-MHz ^1H NMR ($CDCl_3$) δ 0.69 (t, J = 7 Hz, 3 H), 0.91 (t, J = 7 Hz, 3 H), 0.95-1.33 (m, 5 H), 1.56-1.68 (m, 3 H), 1.91-2.15 (m, 3 H), 2.09 (s, 3 H), 2.47-2.73 (m, 3 H), 2.65 (s, 3 H), 2.90 (s, 1 H), 2.93-3.25 (m, 6 H), 3.36-3.52 (m, 2 H), 3.69 (s, 1 H), 3.76 (s, 6 H), 3.69-3.84 (m, 1 H), 3.90 (s, 3 H), 4.02 (br t, 1 H), 5.32 (s, 1 H), 5.35 (d, J = 11 Hz, 1 H), 5.93 (d, d, J = 11, 4 Hz, 1 H), 6.00 (s, 1 H), 6.98 (s, 1 H), 6.98 (t, J = 7 Hz, 1 H), 7.09 (t, J = 7 Hz, 1 H), 7.24 (d, J = 9 Hz, 1 H), 7.36 (d, J = 8 Hz, 1 H), 9.05 (s, 1 H), 9.61 (s, 1 H); 67.9-MHz ^{13}C NMR ($CDCl_3$) δ 175.32, 171.43, 170.70, 156.29, 151.78, 134.76, 134.41, 130.52, 128.29, 126.48, 124.41, 123.92, 121.36, 119.70, 118.28, 117.91, 111.14, 110.39, 94.32, 82.83, 80.00, 65.51, 60.60, 56.08, 55.98, 53.25, 52.60, 52.01, 51.91, 50.74, 48.81, 43.78, 42.80, 39.20, 38.47, 38.19, 33.63, 31.74, 30.53, 29.63, 27.33, 21.00, 11.20, 7.45; mass spectrum, m/z (relative intensity) 794 (M^+, 3), 793 (1), 736 (0.4), 636 (0.6), 528 (0.3), 340 (0.4), 339 (1), 338 (0.3), 282 (0.6), 254 (0.5), 240 (1), 227 (1), 226 (4), 225 (0.5), 213 (1), 212 (7), 199 (7), 198 (2), 197 (0.4), 183 (1), 182 (0.7), 171 (0.5), 170 (0.9), 169 (3), 168 (1), 156 (0.5), 155 (3), 154 (2), 149 (0.8), 142 (0.9), 141 (7), 140 (3), 138 (2), 135 (2), 127 (5), 126 (5), 125 (1), 113 (9), 112 (6), 111 (3), 108 (1), 107 (1), 106 (1), 105 (1), 101 (1), 100 (2), 99 (15), 98 (7), 97 (5), 96 (1), 93 (1), 92 (6), 91 (7), 88 (4), 87 (2), 86 (24), 85 (43), 84 (42), 83 (10), 82 (3), 81 (1), 73 (1), 72 (5), 71 (71), 70 (13), 69 (13), 68 (2), 67 (2), 65 (2), 60 (1), 59 (2), 58 (6), 57 (100), 56 (20), 55 (22); exact mass calcd 794.425, found 794.404.

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Palladium-Catalyzed Synthesis of Alkynylamino Nucleosides. A Universal Linker for Nucleic Acids

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A method for attaching alkynylamino "linkers" to nucleosides and nucleotides is described. Protected or unprotected alkynylamines are coupled to iodonucleosides in dimethylformamide using a 1:2 mol ratio of tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide, a catalyst system superior to the standard system using palladium(II) species. The resulting alkynylamino nucleosides are useful for enzymatic or chemical labeling of all four bases of DNA.

In connection with our program on automated sequencing of DNA, we wanted to prepare chain-terminating,[1] fluorescence-tagged substrates for DNA polymerases.[2] One critical feature of these substrates is the "linker" or group which covalently attaches the fluorescent moiety to the nucleotide substrate without interfering with enzymatic processing of the molecule. Although several linkers are known which meet these requirements,[3] no *general* method is currently available for attaching fluorescent dyes or other reporters[4] to all of the nucleotides found in DNA. Since acetylenes are small and can be attached to some aromatic rings under mild conditions, preparation of nucleosides with alkynylamino groups linked to the heterocyclic ring was investigated.

Robins and Barr[5] had shown that 5-iodouridines with protected hydroxy groups can be coupled with a variety of non-nitrogenous terminal alkynes by treatment with bis(triphenylphosphine)palladium(II) dichloride and copper(I) iodide in warm triethylamine.[6] Attempts to couple propargylamine with unprotected 5-iodouridine under these conditions failed, at least in part because of the insolubility of the iodonucleoside in triethylamine. After some experimentation, it was discovered that couplings between iodonucleosides and alkynylamines could be effected in dimethylformamide by using copper(I) iodide and a palladium(0) catalyst.[7] Specifically, treatment of a

(1) A "chain terminating" substrate for a polymerase is one which, when incorporated onto the 3'-end of an oligonucleotide, blocks enzymatic chain extension of the oligonucleotide. The chain terminators used most often for DNA sequencing are 2',3'-dideoxynucleoside 5'-triphosphates: Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.

(2) Prober, J. M.; Trainor, G. L.; Dam, R. J.; Hobbs, F. W.; Robertson, C. W.; Zagursky, R. J.; Cocuzza, A. J.; Jensen, M. A.; Baumeister, K. *Science* **1987**, *238*, 336.

(3) (a) An allylamino linker has been attached to C5 of uridine: Langer, P. R.; Waldrop, A. A.; Ward, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6633. An alkylamino linker has been attached to N4 of cytidine and N6 of adenosine: Gebeyehu, G.; Rao, P. Y.; Soochan, P.; Simms, D. A.; Klevan, L. *Nucleic Acids Res.* **1987**, *15*, 4513. (c) Derivatives of 3' α-aminonucleoside triphosphates were reported to function as alternative substrates, but the chain terminating species present in these experiments was not unambiguously identified: Krayevsky, A.; Kukhanova, M.; Azhayev, A.; Chidgeavadze, Z.; Beabealashvilli, R. *Nucleic Acids Res. Symposium Ser.* **1984**, *14*, 283. Bililashvili, R. Sh.; Chidzhavadze, Z. G.; Kraevskii, A. A.; Kukhanova, M. K.; Atrazhev, A. M.; Azhaev, A. V.; Kutateladze, T. V. *Biopolim. Kletka* **1985**, *1*, 293-307.

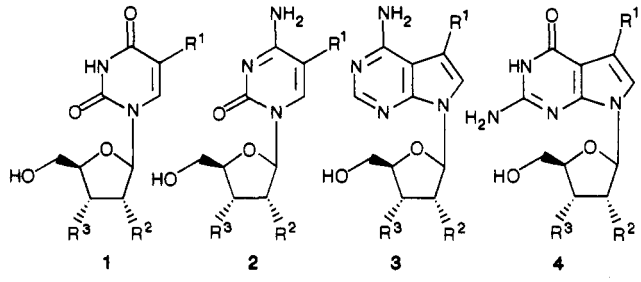
(4) A "reporter" or "reporter group" is a group placed in or attached to a nucleic acid which creates a signal permitting a small quantity of the nucleic acid to be detected. Useful reporters include radioactive isotopes, biotin, and fluorescent dyes. As little as 10^{-18} mol of some of these reporters can be detected.[2]

(5) (a) Robins, M. J.; Barr, P. J. *Tetrahedron Lett.* **1981**, *22*, 421. (b) Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854. (c) Similar products had also been prepared by palladium-catalyzed coupling of alkynylzinc halides to iodouridines: Vincent, P.; Beaucourt, J. P.; Pichat, L. *Tetrahedron Lett.* **1981**, *22*, 945.

(6) This type of coupling was first reported by: Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett* **1975**, 4467.

0022-3263/89/1954-3420$01.50/0

Table I. Preparation of Alkynylamino Nucleosides 1–4



1 2 3 4

	R^1	R^2	R^3	method[a]	yield, %
1a	$C{\equiv}CCH_2NHCOCF_3$	H	H	A	77
1b	$C{\equiv}C(CH_2)_3NHCOCF_3$	H	H	B	80
1c	$C{\equiv}C(CH_2)_{10}NHCOCF_3$	H	H	C	73
1d	$C{\equiv}CCH_2NH_2$	H	H	D	*c*
1e	$C{\equiv}C(CH_2)_3NH_2$	H	H	D	64[c]
1f	$C{\equiv}CCH_2NHCOCF_3$	H	OH	A	81
1g	$C{\equiv}CCH_2NHCOCF_3$	OH	OH	A	80
2a	$C{\equiv}CCH_2NHCOCF_3$	H	H	B	90
2b	$C{\equiv}CCH_2NHCOCF_3$	H	OH	B	74
3[b]	$C{\equiv}CCH_2NHCOCF_3$	H	H	B	92
4[b]	$C{\equiv}CCH_2NHCOCF_3$	H	H	B	78

[a] Method A: 10 mmol of iodonucleoside (R^1 = I); 2.5 mol % Pd(0), 5 mol % CuI, ca. 24 h (with polymeric triphenylphosphine in workup). Method B: 2 mmol of iodonucleoside, 10 mol % Pd(0), 5 mol % CuI, ca. 4 h. Method C: same as method B except 24 h. Method D: same as B except 50 mol of CuI. [b] These iodopurines were prepared by A. J. Cocuzza.[16] [c] These materials could not be completely purified by chromatography.

solution of 5-iodo-2′,3′-dideoxycytidine (2.00 mmol) with copper(I) iodide (0.40 mmol), triethylamine (4.00 mmol), *N*-propargyltrifluoroacetamide (6.00 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.20 mmol) in 10 mL of dimethylformamide for 4 h effected complete conversion of the iodonucleoside to the akynylamino derivative **2a**. The triethylammonium hydroiodide byproduct was then neutralized by adding the bicarbonate form of AG1 X8 resin (ca. 6 mequiv), 10 mL of methanol, and 10 mL of dichloromethane and stirring for 30 min.[8] The reaction mixture was filtered, concentrated (finally at 45 °C, 1 mm), and purified by silica gel chromatography (10–20% methanol in dichloromethane) to afford a 90% yield of nucleoside **2a**. The other alkynylamino nucleosides shown in Table I were prepared similarly. The ^{13}C and ^1H NMR spectra of products **1–4** consisted simply of a superimposition of the spectra of the starting materials minus the acetylenic hydrogen and with a 29–43 ppm downfield shift of the nucleoside carbon which was originally attached to iodine. Their UV spectra exhibited an expected red-shift in the absorption of the longest wavelength band and, in some cases, more complex features: **1a**, 290 and 229 nm; **2a**, 296, 238, and 212 nm; **3a**, 279, 238, and 203 nm; and **4a**, 291, 273, 239, 218, and 201 nm.

This coupling methodology has the following advantages: (a) Alkynylamino linkers can be attached to analogues of all four nucleotides found in DNA. 7-Deazapurines **3** and **4** are used in place of ordinary purines to permit linker attachment to a position compatible with polymerase activity. (b) The amino function of the alkynylamine can be protected with a variety of groups or left unprotected.[9] (c) The reaction occurs under milder conditions than the palladium(II)-catalyzed reactions reported previously. (d) Using this palladium(0)–copper(I) catalyst system, TLC and NMR showed that no side products were generated by cyclization between the acetylene and any of the neighboring heteroatoms on the acetylene or the heterocycle (N4 or O4 of pyrimidines, N6 or O6 of 7-deazapurines).[10]

The ratio of palladium(0) to copper(I) is important to the success of this reaction. For example, when a 1:1 mol ratio of copper(I) to palladium(0) is used, no reaction occurs. When a 3:1 or greater ratio is used with iodouridine **1a** (R^1 = I), the reaction mixture turns black, the starting iodide is consumed exceptionally rapidly, and several products in addition to **1a** are observed by TLC. When a 2:1 ratio is used with the same substrate, the pale yellow reaction mixture remains homogeneous and a single nucleoside product is generated. In most cases, substrates were initially investigated with 10 mol % palladium catalyst and 20 mol % copper(I) iodide. When the alkynylamine is unprotected, however, reactions with a 2:1 ratio of copper to palladium are impractically slow. In these cases a 5:1 ratio is preferable.[11] Smaller amounts of catalysts can also be used. For example, 5-iodo-2′-deoxyuridine is completely converted to **1f** with 1 mol % tetrakis(triphenylphosphine)palladium(0) and 2 mol % copper(I) iodide in 48 h. Triphenylphosphine on polystyrene (5 equiv per copper) can be added to deactivate the catalysts while working up larger scale reactions.

One interesting feature of this coupling reaction is that all of the iodonucleosides investigated react at approximately the same rate. Since the rate of oxidative addition of palladium(0) to some aryl–halogen bonds increases as the aromatic ring becomes increasingly electron-deficient,[12a] oxidative addition is probably not the rate-determining step in these couplings.[12b]

Many of the alkynylamino nucleosides in Table I have been converted to the corresponding 5′-triphosphates by the method of Ruth.[13] The resulting triphosphates with trifluoroacetyl groups have been deprotected with ammonium hydroxide and selectively acylated at the linker nitrogen without affecting any of the other nucleophilic sites found in these nucleosides. These nucleoside triphosphate derivatives have been found to be efficient alternative substrates for a number of polymerases.[2,14] In many cases, the presence of the alkynylamino linker and groups attached to the linker nitrogen have *negligible effect* on the rate at which these nucleotides are utilized as alternative substrates by polymerases. Haralambidis[15a]

(7) In all such coupling reactions, a palladium(0) species presumably oxidatively inserts into the carbon–iodine bond. When a bis(triphenylphosphine)palladium(II) salt is used as the palladium(0) source, only two strong transition-metal ligands are introduced with each palladium. When tetrakis(triphenylphosphine)palladium(0) is used, four strong transition-metal ligands are introduced with each palladium. Ligand stoichiometry, rather than initial palladium oxidation state, probably accounts for the improved properties of this new catalyst system.

(8) Otherwise the triethylamine hydroiodide coelutes with the alkynylamino nucleoside product during column chromatography.

(9) In this case, it is difficult to completely remove all of the transition metal catalysts from the product by column chromatography.

(10) Robins and Barr[5a,b] found that the desired 5-alkynyluracil products were partially converted to furano[2,3-*d*]pyrimidin-2-one side products with the standard palladium(II)–copper(I) catalyst system.

(11) It is tempting to speculate that these coupling reaction work best when copper(I) removes two triphenylphosphine ligands from tetrakis(triphenylphosphine)palladium(0). When a large excess of an alkyne with a free amino group is present, the need for more copper(I) could be explained by competition between these ligands.

(12) (a) Fitton, P.; Rick, E. A. *J. Organomet. Chem.* **1971**, *28*, 287. (b) This hypothesis is supported by the observation that the reaction rate appears to be less than first order in iodonucleoside under some circumstances.

(13) Ruth, J. L.; Cheng, Y.-C. *Mol. Pharmacol.* **1981**, *20*, 415.

(14) Complete details on the synthesis and enzymatic incorporation of various labeled and unlabeled alkynylamino nucleotide triphosphates will be published in due course.

(15) (a) Haralambidis, J.; Chai, M.; Tregear, G. W. *Nucleic Acids Res.* **1987**, *15*, 4857. (b) Gibson, K. J.; Benkovic, S. J. *Nucleic Acids Res.* **1987**, *15*, 6455. These workers used procedures similar to those of Robins[5a] to couple protected propargylamine to 5-iodo-2′-deoxyuridine.

(16) Cocuzza, A. J. *Tetrahedron Lett.* **1988**, *29*, 4061.

and Gibson[15b] have also recently shown that a 5-(propargylamino)-2'-deoxyuridine can be incorporated into an oligonucleotide hybridization probe and labeled with a fluorescent dye or a photoactivable cross-linking agent.

In summary, alkynylamino linkers can be easily attached to a variety of nucleotides in a manner compatible either with enzymatic incorporation into DNA or with chemical synthesis of hybridization probes. The alkynylamino group, therefore, can be considered to serve as a "universal linker" for attaching reporters to nucleic acids.

Acknowledgment. The assistance of my colleagues G. L. Trainor, A. J. Cocuzza, M. A. Jensen, W. A. Nugent, and P. N. Confalone and the technical assistance of L. A. DeAngelis is gratefully acknowledged.

Registry No. 1 (R^1 = I, R^2 = R^3 = H), 105784-83-6; 1 (R^1 = I, R^2 = H, R^3 = OH), 54-42-2; 1 (R^1 = I, R^2 = R^3 = OH), 1024-99-3; **1a**, 114748-60-6; **1b**, 115899-42-8; **1c**, 115899-44-0; **1d**, 115899-46-2; **1e**, 115899-45-1; **1f**, 115899-40-6; **1g**, 120609-05-4; **2** (R^1 = I, R^2 = R^3 = H), 114748-57-1; **2** (R^1 = I, R^2 = H, R^3 = OH), 611-53-0; **2a**, 114748-58-2; **2b**, 115899-38-2; **3**, 114748-71-9; **3** (R^1 = I, R^2 = R^3 = H), 114748-70-8; **4**, 114748-68-4; **4** (R^1 = I, R^2 = R^3 = H), 114748-67-3; $HC{\equiv}CCH_2NHCOCF_3$, 14719-21-2; $HC{\equiv}C(CH_2)_3NHCOCF_3$, 115899-41-7; $HC{\equiv}C(CH_2)_{10}NHCOCF_3$, 115899-43-9; $HC{\equiv}CCH_2NH_2$, 2450-71-7; $HC{\equiv}C(CH_2)_3NH_2$, 15252-44-5; CuI, 7681-65-4; $Pd(PPh_3)_4$, 14221-01-3.

Synthesis of γ-Spirolactones and γ-Spirolactams. Diels–Alder Adducts Based on a 9,10-Dihydro-9,10-ethanoanthracene Structure

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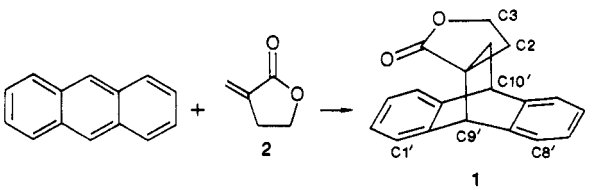
Syntheses of a variety of γ-spirolactones and γ-spirolactams derived from Diels–Alder adducts of anthracene are described. Alkylation of the anion of nitrile 8 with a variety of electrophilic agents affords either C2,C3-substituted γ-spirolactones or C2- or C2,C3-substituted γ-spirolactams. Hydrogenation of 8 affords C2-substituted γ-spirolactams. Changes in relative stereochemistry occur during synthesis of the spirolactones, product stereochemistry being dependent upon temperature and reaction time. A sequence of reactions involving aldol-like condensations and reversible ring closures is suggested to account for the observed stereoselectivities.

Introduction

Spirolactones and spirolactams having the spiro conjunction at the ene terminus of a Diels–Alder adduct are of interest because, as masked exocyclic double bonds,[1] they allow selective protection and deprotection of the latent and possibly labile ene during synthetic manipulation. In addition, the chiral recognition properties of these relatively rigid compounds are of interest in other contexts, for we earlier noted that the enantiomers of **1** are separable on the (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine stationary phase.[2] It was this observation that prompted our initial interest in these compounds. Originally, racemic **1** was prepared by treatment of anthracene with α-methylene-γ-butyrolactone, **2** (Scheme I). The yield of this adduct is low, with polymeric materials being the major products. *exo*-Methylene lactones such as **2** are relatively unavailable, so this synthetic approach to spirolactones or spirolactams is unattractive.

Thebtaranonth's synthesis of spirolactones from the Diels–Alder adduct of anthracene and methacrylate provides the basic strategy for the synthesis of the spirolactones and spirolactams used in this study.[3] Owing to the greater basicity of the carbonyl oxygen and the presence of the NH, which might serve as an additional site for hydrogen bonding, we suspected that spirolactams would resolve more readily on CSP **1** then the corresponding spirolactone. Accordingly, our initial efforts were directed at the synthesis of spirolactams.[4]

Scheme I



Results and Discussion

Spirolactam Synthesis. Thebtaranonth et al. alkylated the enolate of ester **3** with epoxides; the initial adducts close spontaneously to afford spirolactones. Attempts to alkylate this enolate with aziridine led to acylaziridines instead of the desired spirolactams. Alkylation of the enolate (LDA, THF, −78 °C) with ethylene dibromide affords adduct **4** in high yield (Scheme II).[5] Treatment of a methanolic solution of **4** with gaseous ammonia in a sealed glass tube affords γ-spirolactam **5** in 50% yield. In a similar fashion, alkylation with 1,3-dibromopropane followed by amination affords δ-spirolactam **7** in 70% yield. The methylene groups of **4** and **6** are not amenable to further substitutions, so only unsubstituted lactams such

(1) For recent reviews on the application of the retro-Diels–Alder reaction to organic synthesis, see: (a) Ichihara, A. *Synthesis* **1987**, 207. (b) Ripoll, J.; Lasne, M. *Synthesis* **1985**, 121. (c) Wiersum, U. E. *Aldrichimica Acta* **1984**, 17, 31. (d) Brown, R. F. C. *Pyrolytic Methods in Organic Chemistry*; Organic Chemistry Monographs; Academic Press: New York, 1980; Vol. 41. (e) Pirkle, W. H.; Gruber, J. V. *Abstracts of Papers*; 20th Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, WI; American Chemical Society: Washington, DC, 1986; Abstract 283. (f) Flash vacuum pyrolysis of several of the Diels–Alder adducts described herein will be described subsequently.

(2) (a) Pirkle, W. H.; Finn, J. M. *J. Org. Chem.* **1981**, *46*, 2935. (b) Pirkle, W. H.; Finn, J. M. *J. Org. Chem.* **1982**, *47*, 4037. (c) Pirkle, W. H.; Finn, J. M.; Hamper, B. C.; Schreiner, J. L.; Pribish, J. *ACS Symp. Ser.* **1982**, No. 185, 245.

(3) Thebtaranonth, Y.; Jenkitkasemwong, Y.; Wajirum, N. *Tetrahedron Lett.* **1979**, 1615.

(4) Gruber, J. V.; Pirkle, W. H. *Abstracts of Papers*; 19th Midwest Chemical Society, Springfield, MO; American Chemical Society: Washington, DC, 1984; Abstract 622.

(5) Ichihara, A.; Nio, N.; Sakamura, S. *Tetrahedron Lett.* **1980**, *21*, 4467.

0022-3263/89/1954-3422$01.50/0