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Synthesis of a hairpin pyrrole–imidazole polyamide conjugate containing a quinone methide precursor and vinyl linking group

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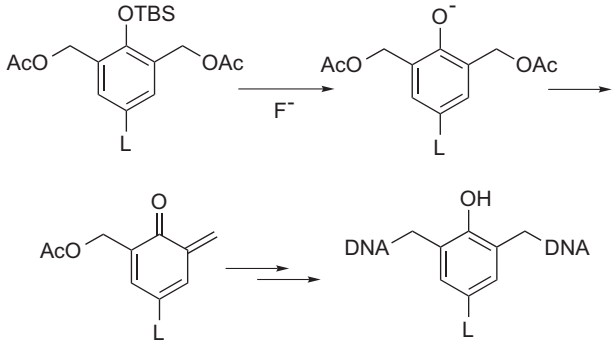
Abstract—Standard procedures for elaborating a quinone methide precursor for conjugation to a DNA ligand was not compatible with the presence of a vinyl group. Instead, an acrylate linker was attached by Heck coupling subsequent to *o*-substitution of the phenolic precursor. This transformation required protection of the phenolic group and use of ethyl acrylate rather than acrylic acid. The presence of the vinyl group also rendered the quinone methide precursor more labile to alkaline conditions than its equivalent saturated derivative and required mild conditions for coupling to the pyrrole–imidazole polyamide.

An important class of antitumor agents acts by cross-linking DNA. Unfortunately, the specificity of these agents is not yet sufficient to avoid many deleterious complications. Considerable effort has therefore been directed toward design and discovery of new compounds that exhibit greater chemical and biological specificity.[1] A variety of natural and synthetic compounds including mitomycin, bizelesin, and merchlorethamine exhibit an intrinsic preference for cross-linking at particular nucleotide sequences.[2] Further selectivity is often gained by conjugating a DNA binding ligand to a reactive appendage, essentially creating an affinity reagent.[3] Pyrrole–imidazole polyamides are especially attract for this purpose due to their predictable association with base pairs through the minor groove of DNA and their compatibility with cellular conditions.[4] Such ligands have been conjugated with alkylating agents related to the cyclopropylpyrroloindole group of CC-1065 and Duocarmycin and to the cross-linking N-mustard chorambucil.[5] A range of linkers have been examined in these conjugates and a relatively rigid *trans* acrylate moiety appears highly effective.[6]

Our laboratory has focused on quinone methides as DNA alkylating and cross-linking agents (Scheme 1).[7] Although an innate selectivity based on product stability



Scheme 1.

favors reaction with the 2-amino group of guanine in the minor groove,[8] a conjugate directing reaction to this site has only now been prepared as reported here. A previous strategy[7c] for adapting a nascent quinone methide for coupling to a sequence-directing ligand and cross-linking to DNA was not appropriate for the acrylate derivative (**9**, Scheme 2). Attempts to hydroxymethylate *ortho* to the phenolic position of 4-hydroxycinnamic acid with formaldehyde under alkaline conditions generated only polymer. Accordingly, substitution at the *ortho* positions was performed first using a Mannich reaction to generate bis(morpholinomethyl)phenol **1** as described by Crisp and Turner.[9] Next, the linker arm was attached and activated for conjugation to the polyamide (Scheme 2).

Keywords: Quinone methide; Pyrrole–imidazole polyamide; DNA alkylation; Minor groove ligand.
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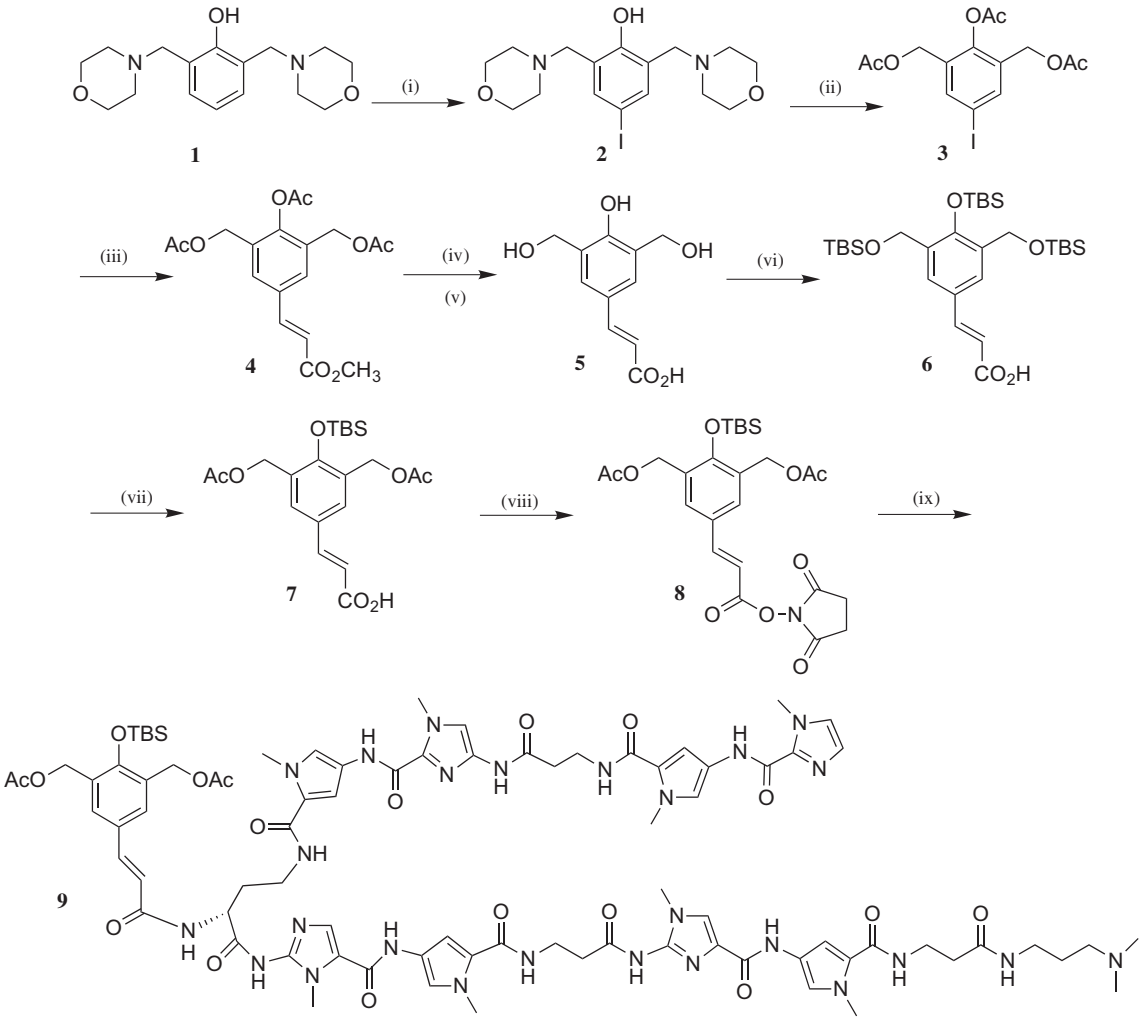

Scheme 2. Reagents and conditions: (i) NaI, chloramine-T, 50 °C; (ii) Ac_2O, AcOH; (iii) $Pd(OAc)_2$, (o-$CH_3C_6H_4)_3P$, methyl acrylate, Et_3N, toluene, 20 h; (iv) 1.25 M H_2SO_4, THF, 80 °C, 3.5 h; (v) LiOH, H_2O, rt, 2 h; (vi) TBS-Cl, imidazole, DMF, 48 h; (vii) $FeCl_3$, Ac_2O, 0 °C; (viii) *N*-hydroxysuccinimide, EDC, CH_2Cl_2, rt; (ix) aqueous DMF/CH_3CN pH 7.5 pyrrole–imidazole polyamide.

Treatment of **1** with NaI/chloramine-T in DMSO yielded the *p*-iodo derivative **2** that was ready for coupling to the linker arm. A palladium-catalyzed Heck reaction proceeded in good yield (**4**, 84%) but only after protecting the phenolic group with acetate (**3**) and using methyl acrylate with $Pd(OAc)_2$, (o-$CH_3C_6H_4)_3P$, and Et_3N (2 equiv) in dry toluene (100 °C).[10] No coupling was detected after **2** was combined with the palladium catalyst and either acrylic acid or its methyl ester under a range of temperatures (55–110 °C) and solvents (DMF, CH_3CN, and H_2O). A similar lack of reaction was observed when acrylic acid was treated with the protected phenol **3** under the anhydrous conditions above. The sensitivity of this reaction is likely based on the inhibitory effects of electron donating groups attached to the aromatic system and the limited quantity of base that could be added in the presence of this quinone methide precursor. Deprotection of **4** by sequential treatment with 1.25 M H_2SO_4 in 75% aqueous THF (80 °C) and aqueous LiOH at room temperature produced 3-[3′,5′-bis(hydroxymethyl)-4′-hydroxyphenyl]-2-propenoic acid **5** in 71% yield. Alternative reflux of the H_2SO_4 solution in attempt to hydrolyze the three esters concurrently led to polymerization of the organic material. The desired trisilyloxy derivative **6** was generated under the standard conditions of excess TBS-Cl and imidazole in DMF at room temperature.[7c] Selective substitution forming the benzylic acetates was achieved by treatment with $FeCl_3$ and acetic anhydride under conditions described by Ganem and Small.[11]

The quinone methide precursor **7** exhibited unusual sensitivity to many of the typical procedures used for its final coupling to DNA ligands. Even stepwise reaction through an *N*-hydroxysuccinimide ester was initially discouraging after a complex mixture of products was detected under routine addition of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) in DMF.[7c] However, the activated ester **8** was successfully isolated in 64% yield when CH_2Cl_2 was substituted for DMF. The presence of the vinyl group also destabilized attachment of the TBS group as evident by its loss after exposure to diisopropylethylamine in either CH_3CN, DMF, H_2O, or their combination under conditions used

to couple the saturated derivative of **8**.[7c] Coupling only proceeded as expected when the activated ester **8** and polyamide were combined under mild conditions of 50% aqueous CH_3CN/DMF (2:1) pH 7.5 at room temperature. The product **9** was isolated by reverse phase (C-18) chromatography and confirmed by electrospray mass spectroscopy.

The set of functional groups required in the desired quinone methide precursor containing an acrylate linker necessitated a new strategy for its construction. Use of a Heck coupling to attach the linker arm subsequent to alkylation of the *ortho* positions will now provide easy access to quinone methide precursors containing a broad array of linkers used to connect with site-directing ligands. Additionally, strongly basic conditions can be avoided during coupling of labile appendages to site-directing ligands without adverse effects.

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References and notes

1. Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723–2795.
2. (a) Tomasz, M.; Das, A.; Tang, K. S.; Ford, M. G. J.; Minnock, A.; Musser, S. M.; Waring, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 11581–11593; (b) Sun, D.; Hurley, L. H. *J. Am. Chem. Soc.* **1993**, *115*, 5925–5933; (c) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Nucleic Acids Res.* **1987**, *15*, 10531–10549; (d) Rink, S. M.; Solomon, M. S.; Taylor, M. J.; Rajur, S. B.; McLaughlin, L. W.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 2551–2557.
3. (a) Goodchild, J. *Bioconj. Chem.* **1990**, *1*, 165–187; (b) Knorre, D. G.; Vlassov, V. V.; Zarytova, V. F. *Design and Targeted Reactions of Oligonucleotide Derivatives*; CRC: Boca Raton, 1994.
4. (a) Sondhi, S. M.; Reddy, B. S. P.; Lown, J. W. *Curr. Med. Chem.* **1997**, *4*, 313–358; (b) Gottesfeld, J. M.; Turner, J. M.; Dervan, P. B. *Gene Expression* **2000**, *9*, 77–91; (c) Dervan, P. B.; Edelson, B. S. *Curr. Opin. Struct. Biol.* **2003**, *13*, 284–299.
5. (a) Tao, Z.-F.; Fujiwara, T.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1999**, *121*, 4961–4967; (b) Wurtz, N. R.; Dervan, P. B. *Chem. Biol.* **2000**, *7*, 153–161; (c) Chang, A. Y.; Dervan, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 4856–4864; (d) Toth, J. L.; Price, C. A.; Madsen, E. C.; Handl, H. L.; Hudson, S. J.; Hubbard, R. B. I.; Bowen, J. P.; Kiakos, K.; Hartley, J. A.; Lee, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2245–2248.
6. (a) Fregeau, N. L.; Wang, Y.; Pon, R. T.; Wylie, W. A.; Lown, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 8917–8925; (b) Tao, Z.-F.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **2000**, *122*, 1602–1608; (c) Bando, T.; Narita, A.; Saito, I.; Sugiyama, H. *Chem. Eur. J.* **2002**, *8*, 4781–4790.
7. (a) Zeng, Q.; Rokita, S. E. *J. Org. Chem.* **1996**, *61*, 9080–9081; (b) Zhou, Q.; Pande, P.; Johnson, A. E.; Rokita, S. E. *Bioorg. Med. Chem.* **2001**, *9*, 2347–2354; (c) Veldhuyzen, W. F.; Pande, P.; Rokita, S. E. *J. Am. Chem. Soc.* **2003**, *125*, 14005–14013.
8. (a) Veldhuyzen, W.; Lam, Y.-f.; Rokita, S. E. *Chem. Res. Toxicol.* **2001**, *14*, 1345–1351; (b) Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 11126–11132.
9. Crisp, G. T.; Turner, P. D. *Tetrahedron* **2000**, *56*, 407–415.
10. **3**: Yield 69%, mp 88–89 °C. ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 6H, $COCH_3$), 2.31 (s, 3H, $COCH_3$), 4.95 (s, 4H, $-CH_2$), 7.72 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 20.4, 20.7, 60.3, 90.5, 131.3, 138.9, 148.0, 168.7, 170.4, HRMS calcd for $C_{14}H_{15}IO_6$ 405.9913, found 405.9906. **4**: The iodobenzene **3** (1.00 g, 2.46 mmol), methyl acrylate (0.480 g, 5.55 mmol), distilled triethylamine (0.43 g, 4.9 mmol), tri-*o*-tolylphosphine (0.075 g, 0.25 mmol), and palladium (II) acetate (0.028 g, 0.13 mmol) in dry toluene (8 mL) were heated at 100 °C under nitrogen for 24 h. Solvent was removed at reduced pressure and the residue extracted with ether (3×10 mL). After removal of ether, the product was purified by silica-gel chromatography using ethyl acetate and hexane (3:7) as solvent. Yield 84%; mp 102 °C; ^{1}H NMR ($CDCl_3$): δ 2.06 (s, 6H, $COCH_3$), 2.33 (s, 3H, $COCH_3$), 3.79 (s, 3H, CO_2CH_3), 5.02 (s, 4H, $-CH_2$), 6.41 (d, 1H, $J = 16$, olefinic), 7.56 (s, 2H); 7.64 (d, 1H, $J = 16$, olefinic); ^{13}C NMR ($CDCl_3$): δ 20.5, 20.8, 51.9, 61.0, 119.1, 129.8, 130.0, 132.9, 143.1, 148.8, 167.1, 168.1, 170.5; HRMS calcd for $C_{18}H_{29}O_8$ 364.1162, found 364.1158. **5**: ^{1}H NMR (MeOH-d_4): δ 4.70 (s, 4H, CH_2), 6.29 (d, 1H, $J = 16$, olefinic), 7.39 (s, 2H), 7.58 (d, 1H, $J = 16$, olefinic); ^{13}C NMR (MeOH-d_4): δ 61.9, 116.2, 127.3, 128.4, 129.1, 146.9, 157.3, 171.1; HRMS calcd for anion $C_{11}H_{10}O_5$ ($M-H^+$) 223.0599, found 223.0606. **6**: Yield 39%; mp 198 °C; ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 12H, $Si(CH_3)_2$), 0.18 (s, 6H, $Si(CH_3)_2$), 0.94 (s, 18H, $C(CH_3)_3$), 1.00 (s, 9H, $C(CH_3)_3$), 4.68 (s, 4H, CH_2), 6.32 (d, 1H, $J = 16$, olefinic), 7.59 (s, 2H), 7.78 (d, 1H, $J = 16$, olefinic); ^{13}C NMR ($CDCl_3$): δ 14.1, 18.4, 18.8, 22.6, 25.9, 60.4, 114.8, 126.4, 127.7, 132.5, 147.7, 150.6, 172.4. HRMS calcd for $C_{29}H_{55}O_5Si_3$ 567.3357, found 557.3362. **7**: Yield 43%; mp 120–121 °C; ^{1}H NMR ($CDCl_3$): δ 0.23 (s, 6H, $Si(CH_3)_2$), 1.03 (s, 9H, $C(CH_3)_3$), 2.12 (s, 6H, $COCH_3$), 5.10 (s, 4H, CH_2), 6.34 (d, 1H, $J = 16$, olefinic), 7.51 (s, 2H), 7.73 (d, 1H, $J = 16$, olefinic); ^{13}C NMR ($CDCl_3$): δ −3.6, 18.8, 21.0, 25.9, 31.0, 61.5, 116.3, 128.0, 129.9, 146.1, 153.5, 170.9, 172.0; HRMS calcd for $C_{21}H_{29}O_7Si$ ($M-H^+$) 421.1683, found 421.1682. **8**: *N*-Hydroxysuccinimide (0.013 g, 0.11 mmol) was added to a solution of acid **7** (0.013 g, 0.031 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was combined with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC, 0.018 g, 0.12 mmol) and stirred for 12 h at room temperature. The product was isolated by silica-gel preparative tlc using EtOAc/hexane (2:3) as solvent. Yield 64%; ^{1}H NMR ($CDCl_3$): δ 0.22 (s, 6H, $Si(CH_3)_2$), 1.02 (s, 9H, $C(CH_3)_3$), 2.12 (s, 6H, $COCH_3$), 2.87 (s, 4H, CH_2), 5.10 (s, 4H, CH_2), 6.49 (d, 1H, $J = 16$, olefinic), 7.53 (s, 2H, Ar–H), 7.86 (d, 1H, $J = 16$, olefinic); ^{13}C NMR ($CDCl_3$): δ −3.5, 18.7, 21.0, 25.7, 25.8, 61.3, 110.4, 127.4, 128.3, 130.1, 149.0, 154.1 162.1, 169.3, 170.7; HRMS calcd for $C_{25}H_{33}LiNO_9Si$ (M+Li) 526.2085, found 526.2101. **9**: A solution of **8** (~0.002 g, 0.004 mmol) in 50 μL of CH_3CN/DMF (2:1) was mixed with polyamide (~0.001 g, 0.001 mmol) and then with aqueous MOPS buffer (250 mM, pH 7.5, 50 μL). The reaction mixture was stirred at room temperature for 10 min and the product was purified by reverse phase (C-18) HPLC. Yield ~20%, ESI-MS calcd for $C_{83}H_{109}N_{26}O_{18}Si$ ($M+H^+$) 1785.8, found 1785.5.
11. Ganem, B.; Small, V. R., Jr. *J. Org. Chem.* **1974**, *39*, 3728–3730.