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Stereoselective synthesis of the 1,N^2-deoxyguanosine adducts of cinnamaldehyde. A stereocontrolled route to deoxyguanosine adducts of α,β-unsaturated aldehydes

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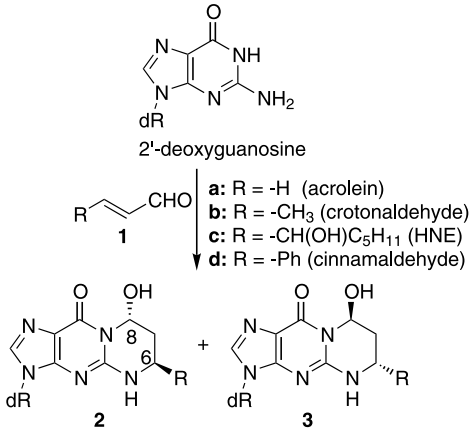
Abstract—α,β-Unsaturated aldehydes (enals) react with deoxyguanosine and have mutagenic potential. For higher enals, the reaction of deoxyguanosine gives diastereomeric 6-substituted 8-hydroxypyrimidopurinone products. These stereoisomers may have different local conformations in DNA, which may have biological consequences. We have developed a stereospecific synthesis of 1,N^2-deoxyguanosine adducts of cinnamaldehyde. The key step is the synthesis is a metal-promoted intramolecular C–H insertion reaction of nitrogen of an enantiomerically pure sulfamate ester. The approach may be general for the stereocontrolled synthesis of this class of DNA adducts and can be applied to the preparation of site-specifically adducted oligonucleotides.

α,β-Unsaturated aldehydes (enals) are ubiquitous in the environment and are products of oxidative damage to polyunsaturated fatty acids.[1,2] They react with DNA bases to form exocyclic adducts and some have been demonstrated to have miscoding potential.[3–6] Crotonaldehyde has been shown to be a rodent carcinogen.[7] As such, enals are an important class of mutagens for which human expose is virtually unavoidable.



Scheme 1. Reaction of enals with deoxyguanosine.

The major products from the reaction of enals with DNA are 1,N^2-hydroxypropano adducts of deoxyguanosine (Scheme 1).[8,9] New stereocenters are generated in the course of this reaction leading to diastereomeric adducts. Within DNA, these diastereomeric adducts may have different local conformations and reactivity which could lead to different biological responses. The reaction of acrolein with deoxyguanosine gives two 8-hydroxy-pyrimidopurinone products (R = H) which interconvert. The regioisomeric 6-hydroxy isomer is also produced.[10,11] For higher aldehydes (R ≠ H), the relative stereochemistry at C6 and C8 is predominately *trans*.[9,12] In recent years, our laboratory has examined DNA adducts of acrolein, crotonaldehyde and *trans*-4-hydroxynonenal (HNE) and find that stereochemistry is critical in defining the chemistry and physical properties of the adducted oligonucleotides.[13–15]

We and others have synthesized N^2-adducts of deoxyguanosine via a nucleophilic aromatic substitution strategy from O^6-protected 2-fluoroinosine (**4**) and an amine analogue of the mutagen (**5**) as outlined in Scheme 2.[16,17] A vicinal diol unit was used as a surrogate for the aldehyde and could be cleaved with sodium periodate after the adduction reaction. A significant advantage of this approach is that the chemistry can be applied to the synthesis of site-specifically adducted

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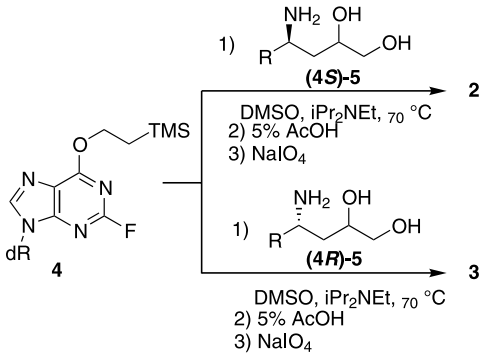


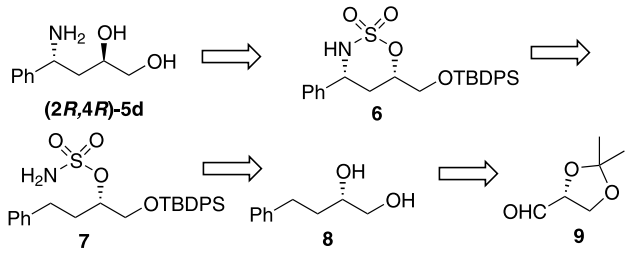
Scheme 2. Stereocontrolled synthesis of 1,N^2-deoxyguanosine adducts of enals.

oligonucleotides via the reaction of the amine with oligonucleotides containing the O^6-protected 2-fluoro-2′-deoxyinosine (**4**).[13,14,18] In the case of crotonaldehyde adducts, a racemic amine (**5b**, R = CH_3) was used and the 6*R*- and 6*S*-diastereomers were separated at the oligonucleotide stage.[19] However, a stereospecific synthesis of one isomer was required to unambiguously establish the absolute stereochemistry. We report here an enantiospecific synthesis of the amino diol unit **5d** (R = Ph), which could potentially be a general approach to the stereocontrolled synthesis of oligonucleotides that contain 1,N^2-deoxyguanosine adducts of enals.

trans-Cinnamaldehyde (**1d**) is a common essential oil and is the principal flavoring component in cinnamon. It is widely used in the flavoring, perfume and cosmetic industries.[20] Cinnamaldehyde has been shown to induce neoplastic transformations in CHO cells and cause lethal mutations in *Drosophila*.[21,22] Interestingly, cinnamaldehyde has also been shown to significantly reduce the spontaneous mutation frequency in *S. typhimurium* TA104 strains.[23] The anti-mutagenic activity required active SOS repair. To our knowledge, no site-specific mutagenesis studies have been reported for 1,N^2-deoxyguanosine adducts of *trans*-cinnamaldehyde. In previously work from our laboratories, we have concentrated on deoxyguanosine adducts of aliphatic enals derived from lipid peroxidation.[13,14] The phenyl ring of the cinnamaldehyde adduct represents a new sub-structure and may be useful in developing structure–activity relationships.

We envisioned controlling the absolute stereochemistry of the critical amino group of **5** via a Du Bois C–H insertion reaction of nitrogen by which enantiomerically pure sulfamate ester **7** gives the cyclic sulfamate **6** (Scheme 3).[24] The sulfamate ester **7** would be derived from selective functionalization of diol **8** which in turn would be available from the isopropylidene derivative of glyceraldehyde (**9**). Both enantiomers of **9** are readily available allowing synthetic access to both enantiomers of amino diol **5**.[25,26]

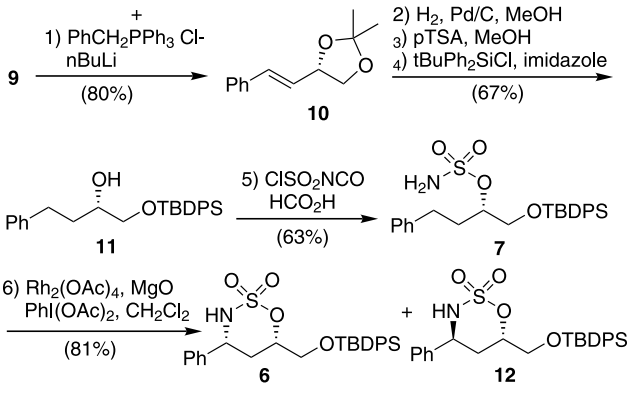
The synthesis began with the Wittig olefination of **9** to give predominantly *trans*-olefin **10** (Scheme 4). The olefin was reduced via catalytic hydrogenation followed by acid



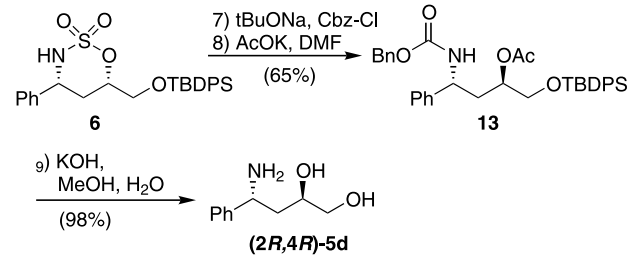
Scheme 3. Retrosynthetic analysis for the synthesis of **5**.

hydrolysis of the isopropylidene and selective protection of the primary alcohol as a *t*-butyldiphenylsilyl ether to give **11** in 67% overall yield. The secondary hydroxyl group of **11** was then converted to the corresponding sulfamate ester **7**.[27] Intramolecular C–H insertion was affected according to the conditions of Du Bois and co-workers to give a 9:1 mixture of oxathiazinane dioxides **6** and **12** in 81% combined yield.[24,28] The products were separable by flash chromatography.

Cyclic sulfamate **6** was converted to the corresponding *N*-Cbz derivative and then treated with potassium acetate to give **13** in which the three functional groups are differentially protected (Scheme 5). Treatment of **13** with tetrabutylammonium fluoride resulted in the corresponding cyclic carbamate which could be hydrolyzed to give (2*R*,4*R*)-**5d**. If the Cbz group was removed first, the *O*-acetyl migrated to the amino group. All three protecting groups could be removed in a single step by treatment of **13** with potassium hydroxide giving the desired product in 98% yield. Using an identical sequence, **12** was converted to (2*R*,4*S*)-**5d**.



Scheme 4. Transfer of stereochemistry for the intra-molecular nitrogen C–H insertion reaction.



Scheme 5. Completion of the synthesis of amino diol **5**.

As outlined in Scheme 2, the stereoisomers of **5d** were individually reacted with 2-fluoro-O^6-(2-(trimethylsilyl)ethyl)-2′-deoxyinosine (**4**) to give the adducted nucleosides after removal of the O^6-trimethylsilylethyl group with mild acid. Sodium periodate cleavage of the diol gave the 1,N^2-(6-phenyl-8-hydroxypropano)-2′-deoxyguanosines (**2d** and **3d**). The conversion of amino diols **5d** to the adducted nucleoside proceeded in 42% overall yield. The adducted nucleoside diastereomers are resolvable by reversed-phase HPLC analysis and this serves as a further check on the stereochemical integrity of the nitrogen insertion products (**6** and **12**) as well the amino diols **5d** (Fig. 1).

We attempted to confirm the stereochemistry of the C–H insertion products **6** and **12** by NOE difference spectra, however, the critical protons were not sufficiently well resolved for the analysis. These compounds were then converted to their corresponding *N*-Cbz derivatives which gave greater spectra dispersion. Unfortunately the NOE difference spectra were ambiguous; it is likely that the six-membered ring of these derivatives adopts a distorted chair conformation, which probably was the basis of the ambiguities. The stereochemistry was therefore confirmed through chemical correlation by converting the amino group of

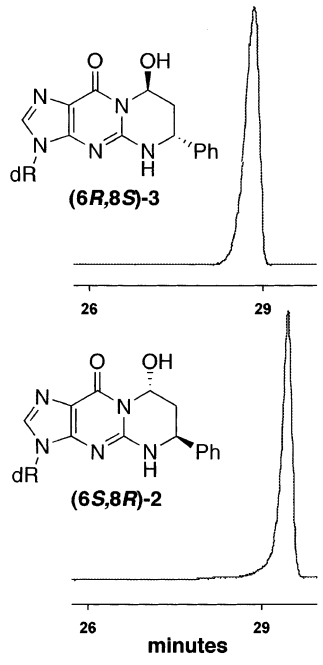
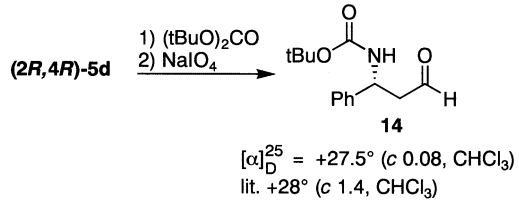


Figure 1. Reversed-phase HPLC analysis of adducted nucleosides **2d** and **3d** monitored at 260 nm.



Scheme 6. Proof of absolute stereochemistry.

(2*R*,4*R*)-**5d** to its BOC derivative followed by periodate cleavage of the diol to the known β-aminoaldehyde **14** (Scheme 6). The sign of the optical rotation of our sample matched that reported by Davis thereby establishing the absolute stereochemistry of (2*R*,4*R*)-**5d** and the relative stereochemistry of the C–H insertions reaction (**6** and **12**).[29]

In summary, we have developed a stereospecific syntheses of the (6*S*,8*R*)- and (6*R*,8*S*)-1,N^2-deoxyguanosine adducts of cinnamaldehyde (**3d** and **2d**, respectively). The key step of the sequence is the Du Bois nitrogen C–H insertion reaction of sulfamate ester **7** to give a separable mixture of cyclic sulfamates **6** and **12**, which were used for the synthesis of **3d** and **2d**. This reaction communicates the stereochemistry for the hydroxyl group, initially obtained from enantiomerically pure D-glyceraldehyde, to the critical amino group. This approach has the potential for a general, stereocontrolled synthesis of 1,N^2-deoxyguanosine adducts of α,β-unsaturated aldehydes. An alternative strategy for the preparation of N^2-modified deoxyguanosine adducts involves the Buchwald–Hartwig cross-coupling of an amine to a suitably protected 2-bromoinosine.[30] However, such an approach would require the preparation of separate phosphoramidite reagents for each adducted oligonucleotide to be studied. A significant advantage of our strategy is that a single oligonucleotide containing the 2-fluoroinosine base (**4**) can be prepared and reacted with a variety of different amines such as (2*R*,4*R*)-**5d** and (2*R*,4*S*)-**5d**, providing a divergent synthetic approach to oligonucleotides with structurally related 1,N^2-deoxyguanosine adducts. In our previous work, we found that stereochemistry is a critical factor in the site-specific mutagenicity of the 4-hydroxynonenal adducts of deoxyguanosine.[6] Also, we have shown that the stereochemistry of the crotonaldehyde and 4-hydroxynonenal adducts has important effects on the ability of these lesions to form interstrand DNA–DNA and DNA–peptide cross-links.[13,14,31] Convenient access to other DNA adducts will allow us to further explore the role of stereochemistry on these and other chemical and biological processes.

Acknowledgements

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15. It should be noted that the designation of the absolute stereochemistry may change depending on the C6-substituent R. For R = -CH_3 and other simple aliphatics, the C6 stereochemistry of **2** and **3** is designated as *R* and *S*, respectively, while for entries **c** and **d** the assignment is reversed.
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27. **7**: Sulfamoyl chloride was prepared by the dropwise addition of formic acid (0.793, 650 μL, 17.2 mmol) to neat chlorosulfonyl isocyanate (2.44 g, 1.5 mL, 17.2 mmol) at 0°C with rapid stirring. Gas was evolved during the addition process. The viscous mixture was stirred for 5 min at 0°C during which time it solidified. Dichloromethane (9 mL) was added and the solution was stirred for 1 h at 0°C then 8 h at 25°C. The reaction mixture was cooled to 0°C and a solution of the **11** (2.37 g, 5.85 mmol) and pyridine (4.60 g, 4.7 mL, 58 mmol) in dichloromethane (4 mL) was added dropwise. The reaction mixture was warmed to 25°C and stirred for 3 h. The reaction was quenched by the successive addition of ethyl acetate (10 mL) and H_2O (5 mL). The biphasic mixture was poured into ethyl acetate (25 mL) and H_2O (10 mL). The phases were separated and the aqueous layer extracted with ethyl acetate (15 mL). The combined organic extracts were washed with saturated brine (2×15 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Purification by flash chromatography on silica gel, eluting with 10% ethyl acetate in hexanes, gave **7** as a clear oil (1.78 g, 63%). $[\alpha]_D$ +15.04° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17–7.65 (m, 15H), 4.62–466 (m, 1H), 4.57–4.60 (br s, 2H), 3.77–3.89 (m, 2H), 2.65–2.75 (m, 2H), 1.94–2.05 (m, 2H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.7, 136.4, 136.3, 130.4, 130.3, 128.8, 128.7, 128.2, 128.1, 126.4, 73.6, 70.9, 35.7, 31.4, 27.4, 19.7.
28. **6** and **12**: To a solution of **7** (0.832 g, 1.75 mmol) in dichloromethane (11.2 mL), were sequentially added Mg_2O (2.07 g, 51.6 mmol), $PhI(OAc)_2$ (0.586 g, 1.82 mmol), and of $Rh_2(OAc)_4$ (0.107 g, 0.243 mmol). The suspension was heated at 40°C with vigorous stirring for 3 h. The reaction mixture was then cooled to 25°C, diluted with dichloromethane (28 mL) and filtered through a pad of Celite. The filter cake was washed with dichloromethane (2×21 mL) and the combined filtrates were evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with 5% ethyl acetate in hexanes to give **6** (614 mg, 73% yield) and **12** (68 mg, 8% yield) as white solids. **6**: mp 43–8°C; $[\alpha]_D = -1.46°$ (*c* 1.46, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26–7.67 (m, 15H), 4.95–4.78 (m, 2H), 4.14 (1H, d, $J = 9.4$ Hz), 3.86 (2H, d, $J = 4.3$ Hz), 2.04–2.10 (m, 2H), 1.03 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.4, 136.0, 135.9, 133.1, 133.0, 130.4, 129.6, 129.3, 128.3, 126.7, 83.7, 65.6, 58.3, 32.5, 27.2, 19.7; **12**: mp 40–42°C; $[\alpha]_D = +24.06°$ (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35–7.65 (m, 15H), 4.85–4.72 (m, 2H), 4.43 (1H, d, $J = 8.5$), 4.14 (m, 2H), 2.30–2.34 (m, 2H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.9, 130.5, 129.4, 128.9, 128.3, 126.7, 82.7, 63.7, 55.5, 30.1, 27.2, 19.6.
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