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Syntheses of Heterocyclic Ethenyl *C*-Nucleosides for Recognition of Inverted Base Pairs within the DNA Triple Helix by Stereoselective Intramolecular Cyclization and Olefin Metathesis

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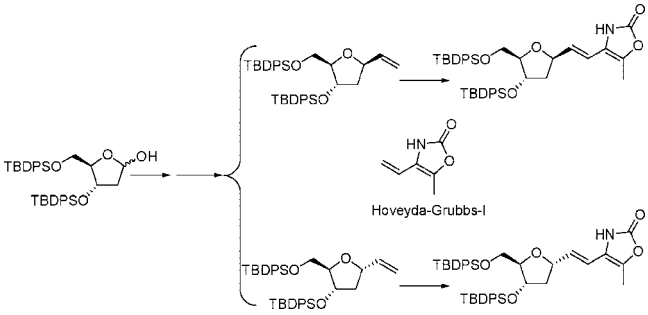

Canonical recognition of gene sequences would allow precise means for specific genetic intervention. Unfortunately, specific recognition of two of the four possible base pairs by triplex-forming oligonucleotide (TFO) as X·T-A and Y·C-G within a triplex currently remains elusive. A series of *C*1-ethenyl nucleosides have been devised and evaluated extensively for stability and specificity by molecular dynamics simulation. A synthesis via olefin metathesis of the unprotected heterocycle and a conveniently prepared anomerically pure *C*1-vinyl 2-deoxyribofuranose is presented as a significant improvement over a previously reported strategy.

The growing interest in gene silencing has made DNA a suitable drug target, and the ability for their direct suppression, deletion, or modification will lead toward more direct strategies for therapy. An essential step toward creating synthetic bioorganic devices that operate upon gene sequences is to have the means for their sequence-specific recognition.

The ability of TFOs to bind duplex target sequences to form intermolecular DNA triple helices offers the possibility of designing compounds with extensive sequence recognition properties that would be useful as antigene agents or tools in molecular biology. One remaining major limitation of this approach is that with natural nucleosides these triplex structures are generally restricted to homopurine−homopyrimidine target sites (Figure 1). TFO strands in parallel orientation H-bonds the polypurine duplex strand of A-T and G-C in the major

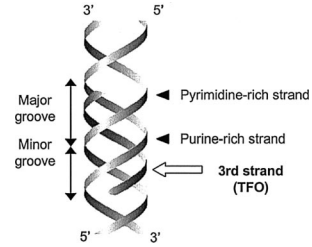

FIGURE 1. DNA triplex; TFO strand parallel or antiparallel.

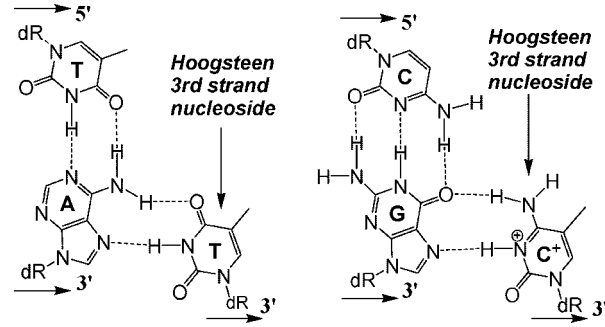

FIGURE 2. T·A-T triplet and 5-MeC$^+$·G-C triplet; Hoogsteen nucleoside is in parallel 3′−5′ orientation to the purine strand.

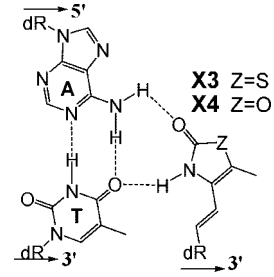

FIGURE 3. X·T-A triplet.

groove (Hoogsteen bonding) as T·A-T and 5-MeC$^+$·G-C triplets, respectively (Figure 2). The 5-methyl derivatization of cytosine increases the pK_a of the H-bonding iminium ion closer to physiological pH, thus significantly improving triplet stability.[1] The current inability to form stable triplexes by recognizing inverted base pairs, T-A and C-G, directly by TFO is a major deterrent in the development of antigene methodologies. The X4 Hoogsteen nucleoside has a sufficient advantage over X3[2] as its heterocyclic π-electron density is closer in dimension to that of the natural nucleobases.

Proposed here is a more general strategy for ethenyl heterocyclic *C*-nucleosides exemplified by the synthesis of prototype 2′-deoxy-*C*-ethenyl β-D-ribonucleoside analogue, X4 (Figure 3). The design of these 2′-deoxy-*C*-ethenyl β-D-ribonucleosides is based upon extensive molecular dynamics evaluations[3−5] which display significantly favorable and specific

(1) Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 3059–3061.
(2) Rothman, J. H. *J. Org. Chem.* **2007**, *72*, 3945–48.
(3) Rothman, J. H.; Richards, W. G. *J. Chem. Soc., Chem. Commun.* **1995**, 1589–90.
(4) Rothman, J. H.; Richards, W. G. *Biopolymers* **1996**, *39*, 795–812.
(5) Rothman, J. H.; Richards, W. G. *Mol. Simul.* **1996**, *18*, 13–42.

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major groove Hoogsteen H-bonding of the T-A base pair orientation. Since TFOs incorporating interspersed α-anomer nucleoside analogues have shown augmented binding stability,[6,7] its preparation is also of interest.

Aryl nucleobases have been the most frequent *C*-nucleoside of interest in the literature. Effective strategies for production of aryl *C*-nucleosides include treatement of Hoffer's α-chlorosugar (3,5-di-*O*-toluoyl-1-chloro-2-deoxy-α-D-ribofuranose) with a diaryl cadmium[8,9] and Heck coupling of aryl triflates or iodides to ribofuranoid glycals.[10−12] One of the more adaptable procedures involves treatment of 3,5-di-*O*-silyl-protected 2-deoxyribonolactone with an aryllithium and subsequent reduction of the resulting hemiacetal with Et_3SiH.[13]

Regarding *C*-ethenyl deoxyribose derivatives, a few synthetic strategies have been reported. The strategy of Takase et al.[14] commences with addition of alkynyllithium reagents to 3,5-di-*O*-benzyl-2-deoxyribofuranose affording diastereomeric mixtures of the corresponding ring-opened alkynyldiols. An intramolecular Nicholas reaction follows giving *C*-alkynyl-3,5-di-*O*-benzyl-2-deoxyribofuranosides with some *β*-selectivity. These may be further modified to *C*-ethenyl derivatives. In another example, a one-pot transformation of unprotected monosaccharides to give styrenyl *C*-glycosides by Horner−Wadsworth−Emmons ring closure and tandem halogenation/Ramberg−Bäcklund sequence proceeds in reasonable yield of the *C*-ethenyl deoxyribose with equal α and *β* anomeric preference.[15] Moreover, the availability of the nucleobase as a sulfonylphosphonate is a requirement. Also available is a six-step intramolecular cyclization strategy allowing *C*-derivatization via Wittig addition at the C-1 of a protected 5-iodo glucofuranose followed by its recyclization to a *C*-ethenyl 2-deoxy-*β*-ribofuranoside.[16] Ring reclosure appears quite steric and requires relatively high temperatures for cyclization; however, yields are high for the *E*-methacrylate example.

An olefin metathesis mediated strategy holds the innate advantages of its functional group tolerance, thermodynamic preference for the *E*-configuration, and mild reaction conditions. For this reason a strategy employing a metathesis of a *C*-vinyl ribofuranose and a vinyl heterocycle was pursued.

A more direct synthesis of the *C*-vinyl ribose component with respect to available strategies was sought. Even if the *C*1-vinyl hemiacetal could be obtained in reasonable yield by addition of a vinyl carbanion strategy to a 3,5-di-*O*-silyl-protected 2-deoxyribonolactone, specific reduction to the *C*1-vinyl ribofuranose would be difficult in the presence of an olefin. Moreover, employment of the HWE-tandem halogenation/Ramberg−Backlund or alkynyllithium addition−intramolecular Nicholas rearrangement strategy could be unnecessarily laborious. However, from a recent work concerning the syntheses of

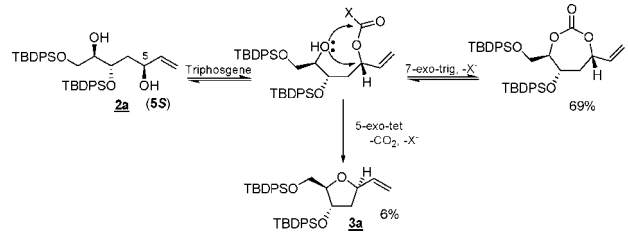
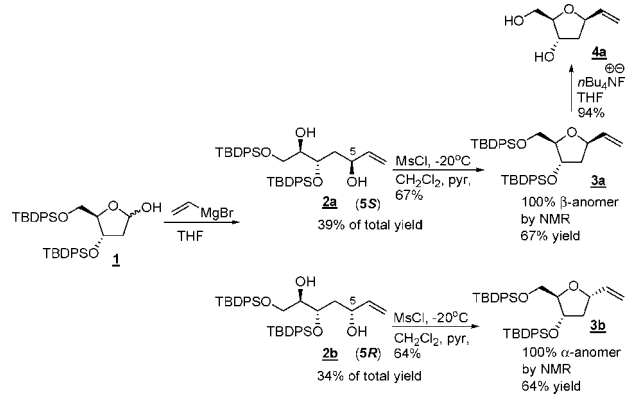


FIGURE 4. *C*1-Vinyl-2-deoxyribofuranose produced by the 5-*exo*-tet cyclization mechanism.

SCHEME 1



7-membered carbonates,[17] by 5-*exo*-tet elimination, the 3,5-bis-*O*-TBDPS protected *C*-vinyl 2-deoxy-*β*-D-ribofuranose, **3a**, was generated stereospecifically as a side product of triphosgene addition to the (5*S*) diol, **2a**.

The formation of the *C*1-vinyl 2-deoxyribofuranose byproduct, **3a**, is most likely due to initial acylation at the less hindered alcohol creating the intermediate (Figure 4) that may follow two possible cyclization pathways. For the circumstances of their research the desired 7-membered cyclic carbonate (Figure 4) is achieved by a 7-*exo*-trig cyclization. However, the byproduct, **3a**, is provided by a 5-*exo*-tet ring closure with inversion of configuration at the reacting center by means of an S_N2-type pathway.[17]

Taking advantage of this observation, the 5-*exo*-tet ring closure was effected more directly by addition of MsCl to the (5*S*) diol **2a** at −20 °C in dichloromethane/pyridine (Scheme 1). This yielded 67% *C*1-vinyl 2-deoxy-D-ribofuranose with near complete stereoinversion to give the *β*-anomer as no α-anomer could be isolated or seen by NMR. This would imply an S_N2-type mechanism. The NMR data for the isolated product fully correspond with that reported by Anderson et al.[17] for the *β*-anomer, **3a**. Analogously, similar treatment of the (5*R*) **2b** diol diastereomer stereospecifically yields the α-anomer, **3b**. The starting (5*S*) **2a**, and (5*R*) **2b** diols are obtained from **1**, the 3,5-bis-*O*-TBDPS protected 2-deoxy-D-ribofuranose[18] (Scheme 1), by treatment with excess vinyl magnesium bromide and separated chromatographically.[17] Deprotection of the silyl moieties was effected with tetra-*n*-butylammonium fluoride yielding the *C*1-vinyl 2-deoxyribofuranose, **4a**. A mixture of the bis-*O*-TBDMS protected diol analogues was also prepared by the same method; however, efficient chromatographic separation was not as satisfactory.

(6) Noonberg, S. B.; Praseuth, D.; Helen, C. *Nucleic Acids Res.* **1995**, *23*, 4042–4049, et al.
(7) Debart, F.; Rayner, B.; Degols, G.; Imbach, J.-L. *Nucleic Acids Res.* **1992**, *20*, 1193–1200.
(8) Chaudhuri, N. C.; Kool, E. T. *Tetrahedron Lett.* **1995**, *36*, 1795–1798.
(9) Pirrung, M. C.; Zhao, X.-D.; Harris, S. V. *J. Org. Chem.* **2001**, *66*, 2067–71.
(10) Hacksell, U.; Daves, G. D. *J. Org. Chem.* **1983**, *48*, 2870.
(11) Czernecki, S.; Dechavanne, V. *Can. J. Chem.* **1983**, *61*, 533.
(12) Coleman, R. S.; Mortensen, M. A. *Tetrahedron Lett.* **2003**, *44*, 1215–1219.
(13) Wichai, U.; Woski, S. *Org. Lett.* **1999**, *1*, 1173–1176.
(14) Takase, M.; Morikawa, T.; Abe, H.; Inouye, M. *Org. Lett.* **2003**, *5*, 625–628.
(15) Jeanmart, S.; Taylor, R. J. K. *Tetrahedron Lett.* **2005**, *46*, 9043–9048.
(16) Egron, D.; Durand, T.; Roland, A.; Vidal, J.-P.; Rossi, J.-C. *Synlett* **1999**, *4*, 435–437.
(17) Anderson, E. A.; Davidson, J. E. P.; Harrison, J. R.; et al. *Tetrahedron* **2002**, *58*, 1943–71.
(18) Walker, J. A.; Chen, J.-J.; Wise, D. S.; Townsend, L. B. *J. Org. Chem.* **1996**, *61*, 2219–2221.

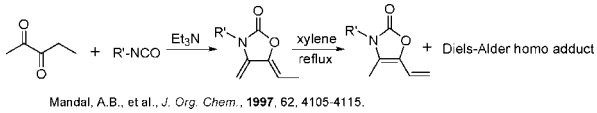
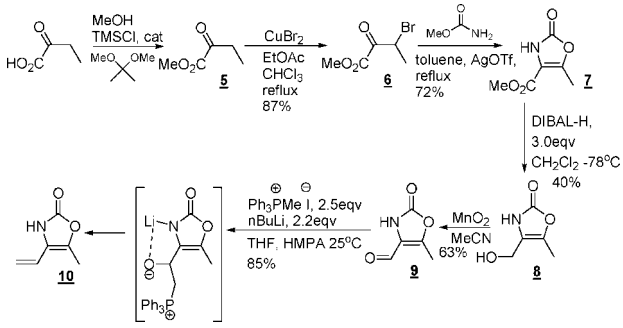
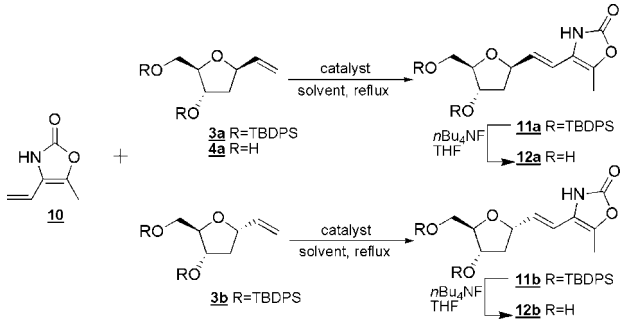


FIGURE 5. 4,5-Dimethylene-2-oxazolidinone preferentially rearranges to 5-vinyl-2-oxazolone.

SCHEME 2



SCHEME 3



To date, syntheses of 4-vinyl oxazolones, thiazolones, or azolones have yet to be reported. The closest related reported structures, *N*-alkyl/aryl 5-vinyl oxazolones, are obtained initially as a 4,5-dimethylene-2-oxazolidinone through condensation of a diacetyl and an alkyl/aryl isocyanate. However, the exocyclic diene preferentially rearranges in refluxing xylene to the 5-vinyl-2-oxazolone and its Diels−Alder homoadduct as the major product (Figure 5).[19]

The 4-vinyl-2-oxazolone component, **10**, reported here is obtained from the 2-oxazolone-4-carboxylic ester, **7**. Through condensation of 4-aryl/alkyl-3-bromo-2-ketoesters with methyl carbamate, 5-aryl/alkyl-2-oxazolone-4-carboxylic esters can be obtained in reasonable yield.[20] To obtain the starting 2-ketobutyric methyl ester, **5**, commercially available 2-ketobutyric acid (Scheme 2) was esterified with methanol in 2,2-dimethoxypropane in the presence of catalytic TMSCl, as a convenient source of anhydrous HCl.[21] In accord with the strategy developed by Hoffmann et al., refluxing the 2-ketoester, **5**, in chloroform/EtOAc with $CuBr_2$ forms the 3-bromo-2-ketoester, **6**, which was subsequently purified and condensed with excess methyl carbamate and silver triflate in refluxing toluene yielding the 2-oxazolone-4-carboxylic methyl ester, **7**. Previous attempts from literature[20] to reduce selectively the ester moiety of the 5-methyl-2-oxazolone-4-carboxylic ethyl ester, without significant over-reduction to the oxazolidinone, have not been entirely successful. Steric interference at the oxazolone 5-position seems to decrease over-reduction with DIBAL-H at room temperature.[20] However, synthesis of the 4-hydroxymethylene derivative, **8**, was achieved in reasonable yield with DIBAL-H at −78 °C. Isolation of the 4-carbaldehyde, **9**, could not be achieved under these conditions or with other general transformation strategies such as $LiAlH_4$−Et_2NH or $LiAlH(O\text{-}t\text{-}Bu)_3$ at 0 °C. Nonetheless, conversion of the 4-hydroxymethylene, **8**, to the aldehyde, **9**, was achieved easily and in high yield with activated MnO_2 in acetonitrile. Yields were relatively modest for this oxidation with use of TPAP/4-methylmorpholine *N*-oxide. Conversion of the aldehyde to the vinyl moiety, **10**, was achieved by Wittig reaction with excess methyltriphenyl phosphorane ylide in THF/HMPA at room temperature. The need for HMPA and relatively high temperature was likely on account of the general insolubility and betaine stabilization due to the proximity of the lithiated amide (Scheme 3).

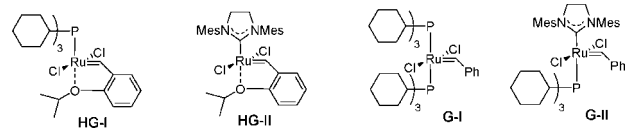


FIGURE 6. Structures of Grubbs and Hoveyda−Grubbs metathesis catalysts.

TABLE 1. Initial Catalyst and Solvent Survey for Olefin Metathesis

*C*1-vinyl ribofuranose	catalyst	solvent	*T* (°C)	yield (%)
3a	HG-I	CH_2Cl_2	40	63
3a	HG-II	CH_2Cl_2	40	18
3a	G-I	CH_2Cl_2	40	10
3a	G-I	toluene	110	5
3a	G-II	CH_2Cl_2	40	14
3a	G-II	toluene	110	5
3b	HG-I	CH_2Cl_2	40	67
3b	HG-II	CH_2Cl_2	40	15
3b	G-I	CH_2Cl_2	40	8
3b	G-II	CH_2Cl_2	40	12
4a	HG-I	CH_2Cl_2	40	NR
4a	HG-II	CH_2Cl_2	40	NR
4a	G-I	CH_2Cl_2	40	NR
4a	G-II	CH_2Cl_2	40	NR

The olefin cross-metathesis final step allows efficient coupling of unprotected nucleoside heterocycle to *C*1-vinyl 2-deoxyribofuranose. Initially, catalysts (Figure 6) and solvents were surveyed for cross-metathesis effectiveness of either the 3′,5′-OH-2-deoxy-*C*1-vinyl-β-D-ribofuranose, **4a**, or 3′,5′-bis-*O*-TBDPS-2-deoxy-*C*1-vinyl-β-D-ribofuranose, **3a**, with 4-vinyl-5-methyl-2-oxazolone, **10** (Table 1). A mixture of 1.0 equiv of reactants at 0.2 M and catalyst (5 mol %) are refluxed in dichloromethane or toluene for 8 h under argon. The solvent was removed and the product purified by preparative silica thin layer chromatography. As seen from Table 1, for the bis-*O*-TBDPS-protected *C*1-vinyl ribofuranose **3a** the best yield of **11a** is achieved with Hoveyda−Grubbs-I (HG-I) catalyst at 63% yield, in comparison to 18% with Grubbs−Hoveyda-II (HG-II) catalyst. A possible reason for the effectiveness of HG-I over HG-II could be explained by steric interference of the mesityl imidazolidinylidene moiety over that of tricyclohexyl phosphine. However, use of Grubbs-I catalyst (G-I) was also not effective for this cross-metathesis as was also Grubbs-II catalyst (G-II).

(19) Mandal, A. B.; Gomez, A.; Trujillo, G. *J. Org. Chem.* **1997**, *62*, 4105–4115.
(20) Okonya, J. F.; Hoffman, R. V.; Johnson, C. *J. Org. Chem.* **2002**, *67*, 1102–1108.
(21) Rodriguez, A.; Nomen, M.; Spur, B. W.; Godfroid, J. J. *Tetrahdron Lett.* **1998**, *39*, 8563–66.

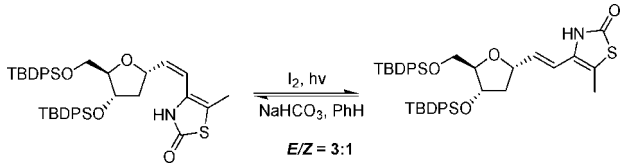


FIGURE 7. Thermodynamic equilibration by photoinitiated iodine-catalyzed isomerization of thiazolone analogue.

In all cases, after equilibrium was achieved to yield maximum product, the only remaining constituents were mainly starting reactants.

The cross-metathesis reaction is effectively quite selective in that the product is consistent with *E*-configuration with no isomer of *Z*-configuration able to be isolated. This is indeed an improvement in comparison to a thermodynamic equilibration by photoinitiated iodine-catalyzed isomerization of a closely related thiazolone analogue of **11b** (Figure 7) yielding an *E*/*Z* ratio of 3:1.[2]

The initial rationale regarding the employment of sterically hindering TBDPS *O*-protecting groups for the *C*1-vinyl-2-deoxyribofuranose reactants is to ensure regioselective cross-metathesis by avoiding formation of its homodimer. However, the unprotected *C*1-vinyl-2-deoxyribofuranose **4a** is found unreactive under metathesis conditions even when refluxing for 48 h. Possibly its vinylidene moiety becomes more durably associated to the catalyst through diol chelation. Nonetheless, metathesis of the 4-vinyl-2-oxazolone, **10**, with bis-*O*-TBDPS-2-deoxy-*C*1-vinyl-α-D-ribofuranose, **3b**, behaves similarly to that of its epimer, **3a**, under these reaction conditions with the best possible yield of **11b** at 67% with Hoveyda−Grubbs-I catalyst (HG-I). Analogously, with the other catalysts yields of **11b** are significantly less, with mainly starting reactants remaining. The bis-*O*-TBDPS-ribofuranose anomers **11a** and **11b** were easily silyl-deprotected with tetra-*n*-butylammonium fluoride yielding their corresponding nucleoside analogues, **12a** and **12b**, respectively. These nucleosides are stable upon exposure to 3% TCA in dichloromethane or 50 mM triazole in THF for 8 h. Irradiation of the anomeric proton produces NOEs consistent with the orientations of each anomer (Figure 8).

Currently, no strategies for convenient preparation of such *C*1-ethenyl 2′-deoxyribonucleosides are available. A means for their synthesis via olefin metathesis of the unprotected hetero-

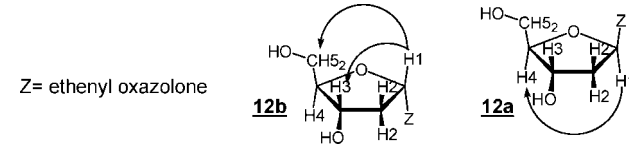


FIGURE 8. Observed NOEs unique to the proposed configurations.

cycle and anomerically pure *C*1-vinyl 2-deoxyribofuranose is presented. Additionally, a convenient means for preparation of a useful precursor for *C*1-nucleosides, diastereomerically pure α-D and β-D *C*1-vinyl-2-deoxyribofuranose, by a stereospecific cyclization is also described.

Experimental Procedures

4-[1-(3,5-Bis-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxy-β-D-ribofuranosyl)-2-*E*-ethenyl]-5-methyloxazol-2-one (11a). In 0.25 mL of dichloromethane both **10** (5.0 mg, 0.046 mmol) and **3a** (28 mg, 0.046 mmol) were dissolved. Then Hoveyda−Grubbs-I (HG-I) (2.8 mg, 0.005 mmol) was added. The reaction contents were refluxed under argon for 8 h, replenishing dichloromethane solvent if necessary. By preparative thin-layer silica chromatography (R_f 0.35, 6:1 toluene, ethyl acetate) 12 mg of white solid was isolated (63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H, N−H), 7.68−7.42 (m, H10, Ar), 7.42−7.23 (m, H10, Ar), 6.26 (d, 1H, CH═C*H*—C═C, J = 15.9 Hz), 5.50 (dd, 1H, C *H*═CH—C═C, J = 16.2, 6.3 Hz), 4.80 (m, 1H, H-1), 4.48 (br d, 1H, H-3, J = 4.8 Hz), 4.03 (m, 1H, H-4), 3.45 (dd, 1H, H-5, J = 11.1, 4.2 Hz), 3.25 (dd, 1H, H-5, J=11.1, 3.0 Hz), 2.05 (s, 3H, C═C—*Me*), 2.03−1.95 (m, 1H, H-2), 1.72−1.63 (m, 1H, H-2), 1.07 (s, 9H, tBu), 0.92 (s, 9H, tBu). ^{13}C NMR (75 MHz, CD_3OD) δ 156, 152, 135.9, 135.7, 133.8, 133.5, 133.2, 130.0, 129.8, 127.9, 127.8, 121.0, 119.5, 114.6, 88.4, 79.1, 76.0, 64.5, 42.9, 27.3, 27.1, 19.6, 19.5, 14.5, 10.6, 1.45. High-resolution FAB$^+$ MS found (M) 717.3285 (MH$^+$), 718.3243; $C_{43}H_{51}O_5NSi_2$ requires (M) 717.3306.

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Supporting Information Available: Detailed experimental procedures for all compounds and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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