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Structure–Activity Relationships Study at the 3′-*N* Position of Paclitaxel. Part 2: Synthesis and Biological Evaluation of 3′-*N*-Thiourea- and 3′-*N*-Thiocarbamate-Bearing Paclitaxel Analogues

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Abstract—The syntheses and preliminary biological evaluation of 3′-*N*-thiocarbamate- and 3′-*N*-thiourea-bearing paclitaxel analogues, **4a–f** and **5a–e**, are described. 3′-*N*-thiocarbamates **4a–e** were found to be more potent than paclitaxel in both the tubulin polymerization assay and the in vitro cytotoxicity assay. Several derivatives of this class such as **4c**, **4d**, and **4e** also exhibited some in vivo activity.

Introduction

Paclitaxel, the active ingredient of the anticancer drug TAXOL® (Bristol-Myers Squibb Co.), has demonstrated antitumor activity against a variety of malignancies that include ovarian, breast, germ cell, lung, and esophageal cancers.[1–3] The taxanes have attracted considerable attention from the research community due to paclitaxel's broad spectrum of clinical activity and its novel mechanism of action, the promotion of microtubule assembly and the stabilization of the resulting microtubules.[4] Numerous reports have appeared describing efforts to identify analogues or formulations that might have increased benefits or advantages over paclitaxel or docetaxel, the two taxanes that are the active ingredients of the drugs approved for clinical use.[5–7]

A number of analogues have incorporated modifications at the side chain 3′-*N* position.[5–7] Replacement of the 3′-*N*-benzamide with a 3′-*N*-tBoc moiety, such as in docetaxel **2a** or 10-acetyl docetaxel **2b**, has been shown to generally result in increased potency in cytoxicity or tubulin polymerization assays when comparing analogues where other modifications remain constant.[8] However, the frequent lack of correlation between antitumor efficacy and relative potency of taxane analogues has stimulated further efforts at 3′-*N* modification. As a result of such efforts, several cytotoxic 3′-*N*-Boc mimics have been reported including *isosteric* 3′-*N*-urea derivatives such as **3**[9a] and the isomeric 3′-carbamate derivatives.[9b] In this report, we describe for the first time, the synthesis and biological evaluation of two novel series of sulfur containing paclitaxel side-chain analogues, namely, 3′-*N*-thiocarbamates **4** and 3′-thiourea derivatives **5** (see Fig. 1).

Chemical Synthesis

The synthetic route employed for the preparation of 3′-free amino derivatives **9a–b** and 3′-*N* *para*-nitrophenyl carbamates **10a–b**, two key precursors for the target molecules **4** and **5**, is shown in Scheme 1. C-13 acylation of the 7-TES baccatin **6**[10] with β-lactams **7a–b**[11] was performed according to the protocol of Holton–Ojima,[12] and provided the desired 3′-*N* protected derivatives **8a–b**. Removal of 3′-*N* protecting group was affected by standard hydrogenation leading to the desired 3′-free amines **9a–b** in 59–94% yield, respectively. Reaction of these amines with *para*-nitrophenyl chloroformate and Hunig's base thus provided the 3′-*N* carbamates **10a–b** in excellent yields.

Scheme 2 shows the syntheses of six 3′-*N*-thiocarbamates derivatives **4a–f**. These analogues were prepared in good to excellent yields by reacting a THF solution of 3′-*p*-nitrophenyl carbamate **10a** or **10b** with a series of butyl/

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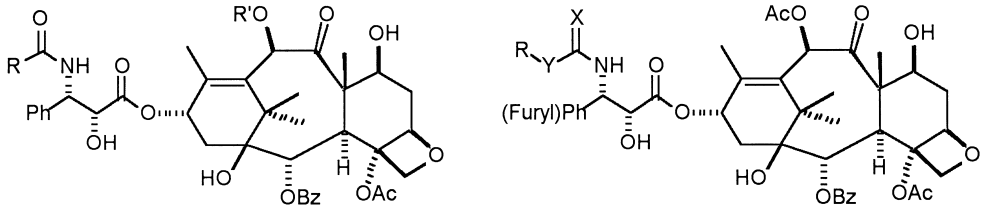
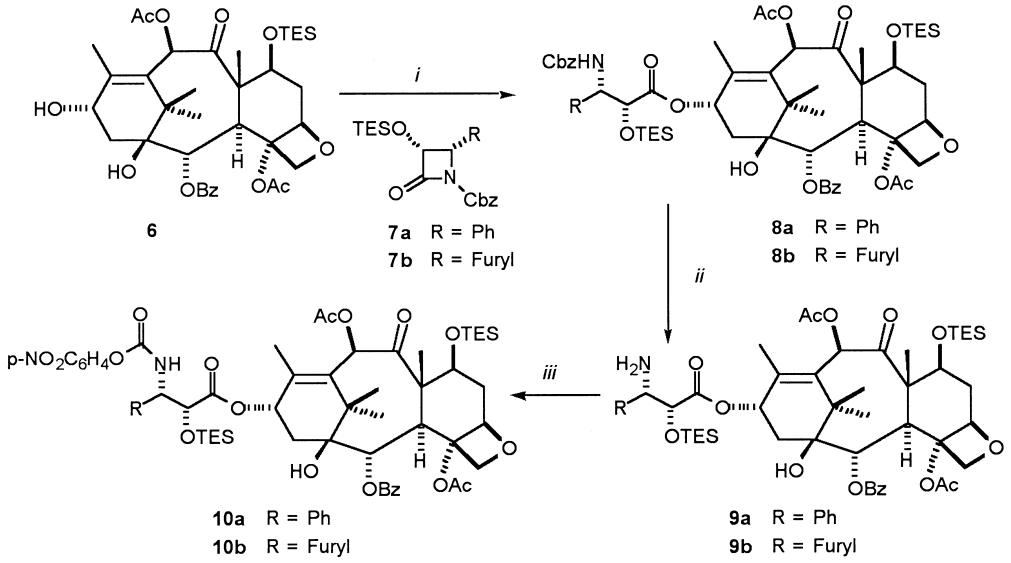
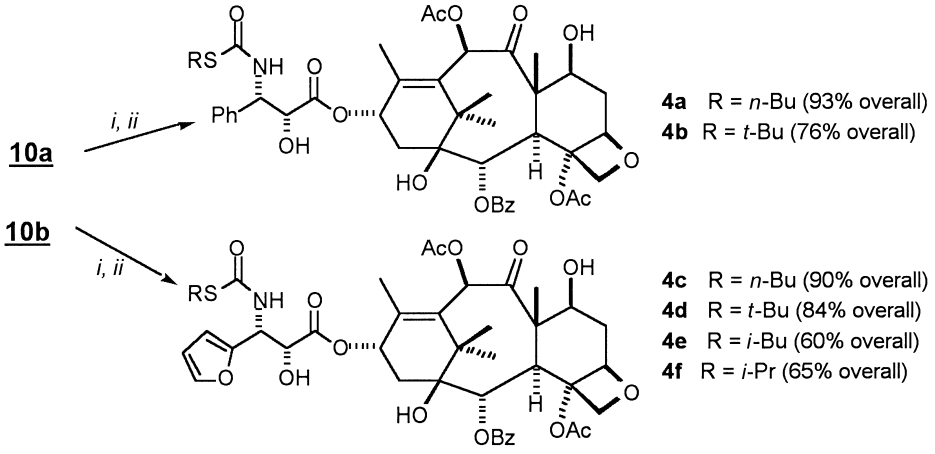


Figure 1.



Scheme 1. Reagents and conditions: (i) LHMDS/THF/**7a**/0 °C, 87% of **8a**; LHMDS/THF/**7b**/0 °C, 80% of **8b**; (ii) 50 Psi H_2/Pd(OH)$_2$/C/EtOAc, 94% of **9a** (60% conversion), or 59% of **9b**; (iii) *p*-$NO_2C_6H_4OC(O)Cl$/$EtPr_2N$/CH_2Cl_2DMAP/0 °C, 97% of **10a**; 93% of **10b**.

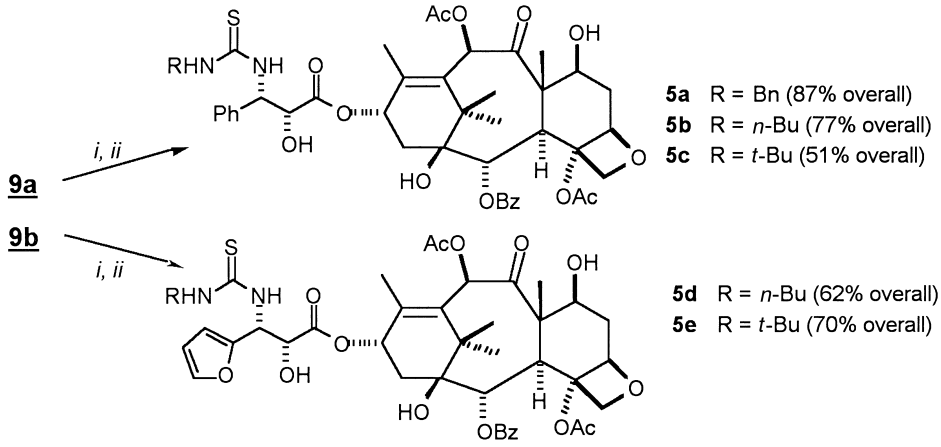


Scheme 2. Conditions: (i) RSNa/THF/0 °C to rt; (ii) 48% HF/Pyridine/CH_3CN/5 °C.

propylthiol sodium salts followed by standard desilylation. The syntheses of the five 3′-*N*-thiourea derivatives **5a–e** were achieved in two steps via acylation of the 3′-amino group in **9a** or **9b** with benzyl, *n*-butyl and *t*-butyl isothiocyanates followed by desilylation (see Scheme 3). Every side-chain analogue described in this manuscript (**4** and **5**) was characterized by its proton, carbon, and high-resolution mass spectra.

Biological Evaluation

A total of eleven 3′-sulfur containing analogues were evaluated in a tubulin polymerization assay[13] and an in vitro cytotoxicity assay against human colon cancer cell lines (HCT-116).[14] Analogues exhibiting better potency than paclitaxel in vitro were further tested in an in vivo screen against murine M 109 lung carcinoma.[15]



Scheme 3. Conditions: (i) RN=C=S/cat. pyridine/C_6H_6; (ii) 48% HF/Pyridine/CH_3CN/5 °C.

As summarized in Table 1, all of six 3′-*N*-thiocarbamate analogues **4a–f** were 2–10 times more potent than paclitaxel in the tubulin polymerization assay. With the exception of **4f**, analogues **4a–e** were found up to 5-fold more cytotoxic than parent paclitaxel. The lack of cytotoxicity of **4f** was surprising. Three of these analogues, **4c–e**, were further evaluated after ip (interperitoneal) administration in an in vivo screen against as ip-implanted M 109 tumor. All three analogues were active (T/C >125%). However, these analogues possessed relatively weaker activities as compared to paclitaxel **1** and 3′-*N*-Boc derivative **2b** in this experiment. Remarkably, several 3′-*N*-thiocarbamates (**4b**, **4d**, **4e**, and **4f**) exhibited excellent in vitro potency against the paclitaxel resistant HCT-116 human colon cancer cell line (see R/S ratios listed in Table 1 for details). The resistance to paclitaxel in this cell line is due to the overexpression of *p*-glycoprotein.

Of the five 3′-*N*-thiourea analogues evaluated, only two 3′-C furyl bearing analogues, **5d** and **5e**, exhibited comparable activity to that of paclitaxel in the tubulin polymerization assay. Disappointingly, analogues **5d–e** were 5-fold less cytotoxic than paclitaxel. The discrepancy observed between these two bioassays may be attributed to the efficiency of drug transport. On the other hand, three 3′-C phenyl containing analogues, **5a–c**, were essentially inactive in both the tubulin and the cytotoxicity assay. The striking different in vitro activity observed between 3′-*N*-urea bearing derivative **3** and its isosteric 3′-*N*-thiourea analogues **5a–e** clearly indicates that the carbonyl moiety attached to the 3′-amino group is a key structural requirement for biological activity, presumably due to its involvement in the intramolecular and/or intermolecular hydrogen bonding.[16]

In summary, we have succeeded in the synthesis of two new series of 3′-*N*-sulfur containing analogues as exemplified by structures **4**[17] and **5**.[18] Our preliminary biological evaluation of these analogues showed that most of the 3′-*N*-thiocarbamate carrying analogues described here demonstrated superior potency in vitro to that displayed by paclitaxel, while the 3′-*N* thiourea bearing derivatives prepared to date possessed reduced potency with respect to paclitaxel in the bioassays tested. The superior

Table 1. In vitro and in vivo activities of side chain analogues **4(a–f)** and **5(a–e)**

Compound	3′-*N* substituent	3′-*C*	Tubulin ratio[a]	Cytotoxicity ratio[b]	IC_{50} (nM) HCT-116 (IC_{50}-*R*/IC_{50}-*S*)	ip M-109 T/C (mg/Kg/inj)[c]
1	C(O)Ph	Ph	1.0	1.0	1.6–6.0 (131)	145–218 (60)
2b	C(O)OBu-*t*	Ph	0.66	0.60	2.4 (12)	177 (32)
3	C(O)NHBu-*t*	Ph	0.72	1.0	4.0 (46.8)	n.d.
4a	C(O)SBu-*n*	Ph	0.6	0.88	5.8 (29)	n.d.
4b	C(O)SBu-*t*	Ph	0.3	0.30	0.9 (4.7)	n.d.
4c	C(O)SBu-*n*	2-Furyl	0.2	0.17	0.3 (21.3)	139 (40)
4d	C(O)SBu-*t*	2-Furyl	0.1	0.30	0.9 (2.2)	134 (1.25)
4e	C(O)SBu-*i*	2-Furyl	0.2	0.28	0.7 (7.7)	145 (50)
4f	C(O)SPr-*i*	2-Furyl	0.2	14.7	32.5 (1.2)	n.d.
5a	C(S)NHBn	Ph	12	>40	>87	n.d.
5b	C(S)NHBu-*n*	Ph	18	21.8	50.2 (64)	n.d.
5c	C(S)NHBu-*t*	Ph	19	~50	96.7 (31)	n.d.
5d	C(S)NHBu-*n*	2-Furyl	1.0	4.6	10.5 (252)	n.d.
5e	C(S)NHBu-*t*	2-Furyl	1.0	4.9	11.3 (40)	n.d.

[a]Ratio of analogue relative to paclitaxel ($EC_{0.01}$). Analogues with ratios < 1 being more potent.
[b]IC_{50}(analogue)/IC_{50}(paclitaxel). Ratios < 1 being more potent. IC_{50}-*R*: IC_{50} values of analogues determined in the paclitaxel resistant HCT-116 human colon cancer cell line. IC_{50}-*S*: IC_{50} values of analogues measured in the paclitaxel sensitive HCT-116 human colon cancer cell line.
[c]Treatment given on days 5 and 8 post-tumor implant.

in vitro potency observed with the 3′-thiocarbamate analogues did not appear to translate into better efficacy in vivo. It is also important to point out that a number of 3′-*N*-thiocarbamates demonstrated impressive potency against resistant HCT-116 cell line in vitro. We believe the results reported herein should further our understanding of paclitaxel side chain SAR.

Acknowledgements

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17. General procedure for the preparation of 3′-thiocarbamate bearing paclitaxel analogues **4a**–**4f**: *p*-Nitrophenyl carbamate **10a/10b** (0.25 mmol, 1 equiv) was dissolved in dry THF (4 mL). To this solution at 0 °C was added a suspension of RSNa (0.33 mmol, 1.3 equiv.) in THF (1 mL). The reaction mixture was stirred at rt for 90 min. At this point, the solvent was removed, the resulting residue was purified with silica gel chromatography (20–30% EtOAc:Hexanes) to provide the corresponding 2′,7-bisTES protected 3′-thiocarbamate paclitaxel intermediate. This intermediate (0.24 mmol) was then dissolved in CH_3CN (5 mL), and treated at 0 °C with pyridine (0.70 mL), followed by 48% HF (2.1 mL). The reaction mixture was kept at 5 °C for 12 h. The reaction mixture was then diluted with EtOAc (75 mL), and washed with 1 N HCl (×1), $NaHCO_3$ saturated solution (×4) and water. The resulting organic phase was dried and concd in vacuo. The residue was chromatographed (40–60% EtOAc:Hexanes) to give the desired final desilylated 3′-thiocarbamate derivative in good to excellent overall yield. ^{1}H NMR of **4a** (300 MHz, $CDCl_3$) δ 8.13–8.10 (m, 2H), 7.64–7.26 (m, 8H), 6.26 (m, 3H), 5.65 (d, J = 7.0 Hz, 1H), 5.60 (m, 1H), 4.93 (d, J = 8.7 Hz, 1H), 4.68 (m, 1H), 4.40 (m, 1H), 4.23 (AB q, J = 8.4 Hz, 2H), 3.79 (d, J = 6.9 Hz, 1H), 3.48 (d, J = 5.0 Hz, 1H), 2.80–0.74 (m, 31H, incl. singlets at 2.36, 2.24, 1.83, 1.68, 1.26, 1.15, 3H each). ^{1}H NMR of **4b** (300 MHz, $CDCl_3$) δ 8.11–8.08 (m, 2H), 7.60–7.32 (m, 8H), 6.24 (m, 3H), 5.65 (d, J = 7.0 Hz, 1H), 5.54 (d, J = 8.7 Hz, 1H), 4.93 (d, J = 8.0 Hz, 1H), 4.65 (s, 1H), 4.40 (m, 1H), 4.22 (AB q, J = 8.5 Hz, 2H), 3.79 (d, J = 7.0 Hz, 1H), 3.52 (d, J = 3.9 Hz, 1H), 2.60–1.14 (m, 31H, incl. singlets at 2.36, 2.23, 1.83, 1.67, 1.26, 1.14, 3H each, 1.34, 9H). ^{1}H NMR of **4c** (300 MHz, $CDCl_3$) δ 8.12–8.09 (m, 2H), 7.59–7.45 (m, 4H), 6.36–6.21 (m, 4H), 5.64 (d, J = 6.9 Hz, 2H), 4.93 (d, J = 8.4 Hz, 1H), 4.73 (d, J = 3.2 Hz, 1H), 4.40 (m, 1H), 4.21 (AB q, J = 8.3 Hz, 2H), 3.80 (d, J = 6.8 Hz, 1H), 3.68 (d, J = 5.6 Hz, 1H), 2.76–1.13 (m, 28H, incl. singlets at 2.38, 2.23, 1.86, 1.66, 1.24, 1.13, 3H each), 0.76 (t, J = 7.1 Hz, 3H). ^{1}H NMR of **4d** (300 MHz, $CDCl_3$) δ 8.11–8.08 (m, 2H), 7.59–7.40 (m, 4H), 6.37–6.17 (m, 4H), 5.64 (m, 2H), 4.94 (d, J = 8.3 Hz, 1H), 4.71 (m, 1H), 4.40 (AB q, J = 8.4 Hz, 2H), 3.80 (d, J = 7.0 Hz, 1H), 3.62 (d, J = 5.5 Hz, 1H), 2.62–1.13 (m, 31H, incl. singlets at 2.39, 2.23, 1.87, 1.66, 1.24, 1.14, 3H each, 1.35, 9H). ^{1}H NMR of **4e** (300 MHz, $CDCl_3$) δ 8.13–8.10 (m, 2H), 7.61–7.42 (m, 4H), 6.39–6.15 (m, 4H), 5.66 (d, J = 7.1 Hz, 2H), 4.94 (d, J = 7.9 Hz, 1H), 4.74 (d, J = 2.1 Hz, 1H), 4.41 (dd, J = 6.7 Hz, J' = 10.8 Hz, 1H), 4.23 (AB q, J = 8.4 Hz, 2H), 3.81 (d, J = 7.1 Hz, 1H), 2.80–0.78 (m, 25H, incl. singlets at 2.38, 2.24, 1.88, 1.68, 1.25, 1.15, 3H each), 0.82 and 0.80, (d, J = 6.7 Hz, 3H each). ^{1}H NMR of **4f** (300 MHz, $CDCl_3$) δ 8.10–8.07 (m, 2H), 7.60–7.38 (m, 4H), 6.35–6.20 (m, 4H), 5.62 (d, J = 7.0 Hz, 1H), 4.90 (d, J = 8.1 Hz, 1H), 4.71 (s, 1H), 4.38 (m, 1H), 4.20 (AB q, J = 8.4 Hz, 2H), 3.78 (d, J = 7.0 Hz,1H), 3.66 (s, 1H), 3.45 (m, 1H), 2.60–1.07 (m, 28H, incl. singlets at 2.36, 2.20, 1.84, 1.64, 1.22, 1.10, 3H each, doublets (J = 6.8 Hz, 3H each) at 1.16, 1.09).
18. Proton NMR spectra of 3-thiourea derivatives **5a**–**5e**: ^{1}H NMR of **5a** (300 MHz, $CDCl_3$) δ 8.05–8.02 (m, 2H), 7.55–7.19 (m, 13H), 6.87 (bs, 2H), 6.23–6.18 (m, 3H), 5.60 (d, J = 7.1 Hz, 1H), 4.84 (d, J = 9.4 Hz, 1H), 4.72 (s, 1H), 4.49 (m, 2H), 4.32 (m, 1H), 4.18 (AB q, J = 9.4 Hz, 2H), 3.72 (d, J = 7.0 Hz, 1H), 2.62–1.07 (m, 22H, incl. singlets at 2.35, 2.18, 1.75, 1.63, 1.17, 1.08, 3H each). ^{1}H NMR of **5b** (300 MHz, $CDCl_3$) δ 8.07–8.04 (m, 2H), 7.60–7.30 (m, 8H), 6.66 (bs, 1H), 6.26 (m, 3H), 5.63 (d, J = 7.0 Hz, 1H), 4.90 (d, J = 8.4 Hz, 1H), 4.80 (s, 1H), 4.36 (m, 1H), 4.20 (AB q, J = 8.4 Hz, 2H), 3.84 (m, 1H), 3.76 (d, J = 6.9 Hz, 1H), 3.13 (bs, 2H), 2.61–1.11 (m, 28H, incl. singlets at 2.35, 2.21, 1.80, 1.66, 1.22, 1.12, 3H each), 0.86 (t, J = 7.2 Hz, 3H). ^{1}H NMR of **5c** (300 MHz, $CDCl_3$) δ 8.09–8.06 (m, 2H), 7.58–7.33 (m, 8H), 6.58 (d, J = 9.1 Hz, 1H), 6.37–6.26 (m, 3H), 6.18 (s, 1H), 5.64 (d, J = 7.1 Hz, 1H), 4.91 (d, J = 7.8 Hz, 1H), 4.85 (s, 1H), 4.39 (m, 1H), 4.22 (AB q, J = 8.5 Hz, 2H),

3.78 (d, $J = 7.1$ Hz, 1H), 3.48 (s, 1H), 2.60–1.13 (m, 31H, incl. singlets at 2.39, 2.21, 1.76, 1.68, 1.25, 1.13, 3H each, 1.39, 9H). ^{1}H NMR of **5d** (300 MHz, $CDCl_3$) δ 8.08–8.05 (m, 2H), 7.58–7.41 (m, 4H), 6.45–6.25 (m, 4H), 6.17 (d, $J = 9.4$ Hz, 1H), 6.00 (bs, 1H), 5.64 (d, $J = 7.1$ Hz, 1H), 4.92 (d, $J = 9.2$ Hz, 1H), 4.83 (d, $J = 2.1$ Hz, 1H), 4.40 (m, 1H), 4.22 (AB q, $J = 8.4$ Hz, 2H), 3.79 (d, $J = 7.0$ Hz, 1H), 3.33 (d, $J = 4.3$ Hz, 1H), 3.26 (bs, 1H), 2.60–0.87 (m, 31H incl. singlets at 2.43, 1.85, 1.67, 1.55, 1.23, 1.12, 3H each, triplet at 0.90, 3H). ^{1}H NMR of **5e** (300 MHz, $CDCl_3$) δ 8.07–8.04 (m, 2H), 7.56–7.41 (m, 4H), 6.45–6.21 (m, 6H), 5.63 (d, $J = 7.1$ Hz, 1H), 4.92 (d, $J = 7.9$ Hz, 1H), 4.85 (s, 1H), 4.40 (m, 1H), 4.21 (AB q, $J = 8.5$ Hz, 2H), 3.79 (d, $J = 7.1$ Hz, 1H), 3.42 (bs, 1H), 2.60–1.11 (m, 31H, incl. singlets at 2.43, 2.22, 1.83, 1.66, 1.23, 1.11, 3H each, 1.39, 9H).