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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1185–1190

Interaction of bulged DNA with leucine-containing mimics of NCS-chrom

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Received 14 September 2005; revised 8 November 2005; accepted 24 November 2005
Available online 10 January 2006

Abstract—Synthesis of chiral spirocyclic helical compounds containing leucine that mimic the molecular architecture of the potent DNA bulge binder obtained from the natural product metabolite NCSi-gb has been accomplished. The interaction between the compounds and DNA was studied by circular dichroism (CD) method. The results suggested that the two synthetic diastereoisomers specifically targeted the bulge site of DNA and induced conformational change of bulged DNA greatly.

Nucleic acids can have richly diverse structures including hairpins, knots, pseudoknots, triple helices, loops, helical junctions, and bulges,[1] among which bulged structure is one of the important motifs in DNA recognition. The extra-unpaired base(s) is capable of either forming complexes with nucleic acid-binding proteins or acting as the binding site for small molecules.[2–4] Interests in developing small molecules that possess specific affinity for bulged sequences[5–7] have been considerably aroused recently. The most promising DNA bulge-specific agent discovered today originated from the work on the enediyne natural product neocarzinostatin chromophore (NCS-chrom, **1**).[8] NCSi-gb **2** (Scheme 1), the metabolite of a general base-catalyzed intramolecular cyclization of NCS-chrom (**1**) in the absence of DNA,[9] can recognize and bind to bulged DNA.[10] In addition, NCSi-gb is able to induce the formation of a DNA bulge by stacking between the base pairs that flank the bulge site.[11]

Based on the analysis of the binding data, NMR, and semiempirical PM3 calculations for NCSi-gb, small molecules capable of preferential binding to DNA bulge sites have been synthesized, with the expectation that such molecules might be used to study the role of bulged structures in nucleic acid function.[5] These synthetic molecules show micromolar affinity for 2 base bulges, which could be increased significantly by subsequent aminoglycosylation.[5] Stimulation of DNA strand slippage synthesis by the bulge binding synthetic agent has also been demonstrated, which exhibited the enhanced effect of the nucleotide repeat slippage during in vitro DNA synthesis.[5d,12]

Considering the central position of amino acids/peptides in the evolution of DNA- and RNA-binding proteins and certain natural products,[13] we postulate that the replacement of amino sugar by amino acids/short peptides in NCSi-gb might help in the binding of bulge sites. Thus, we set about synthesis of conjugates of amino acids and spiroalcohol (Scheme 2).

The synthesis of spiroalcohol **3** was carried out to Ref. 5a by some modification. A Boc-protected L-leucine **4** was subjected to coupling with racemic **3** to give a mixture of diastereoisomers, which were separated by silica gel chromatography, and then subjected to deprotection to furnish diastereomers **7** and **8**.[14,15] The structures of **5** and **6** were fully assignable by NMR and CD spectroscopy and X-ray analysis (compound **5**,[16] Fig. 1). The stereochemistry of **7** and **8** was consistent with the results of CD spectra.

The CD spectra of **7** and **8** in TE buffer (Fig. 2) displayed absorptions at 220, 250, and 280 nm. These peaks are associated with the corresponding π–π* transitions in the UV spectra. Accordingly, the CD of **7** (solid line) showed a positive Cotton effect (CE) at 246 and a negative CE at 220, 300 nm (weak). The CD of **8**

Keywords: Bugled DNA; Neocarzinostatin; Circular dichroism; Drug–DNA interaction.
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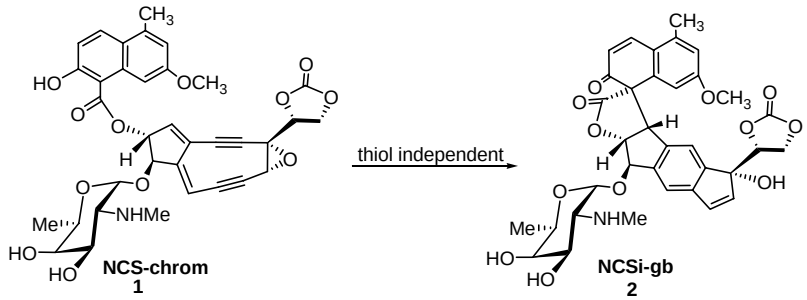
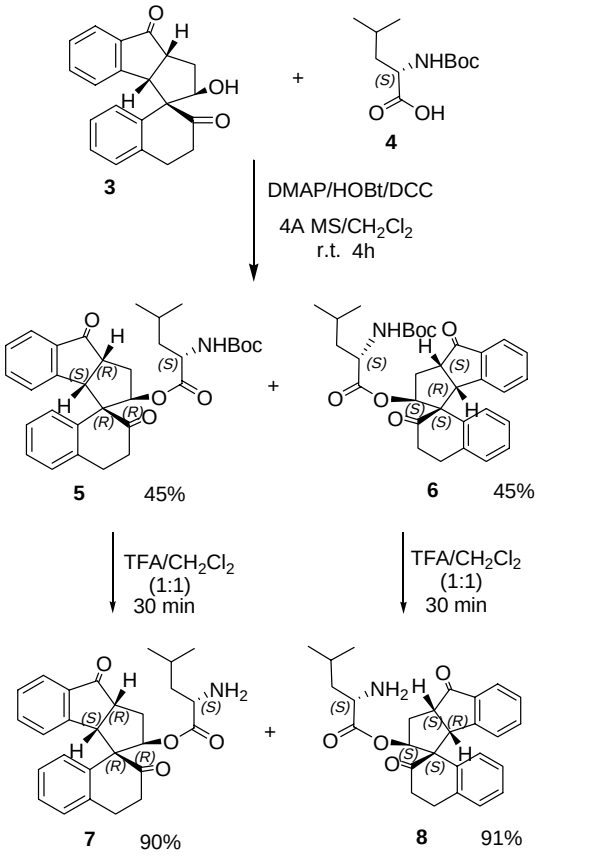
doi:10.1016/j.bmcl.2005.11.084



Scheme 1. Activation cascade of neocarzinostatin chromophore in the absence of thiol.



Scheme 2. Synthesis of chiral spirocyclic helical compounds containing leucine.

(dashed line) showed positive CE at 220, 280 and negative CE at 249 nm, which was almost complementary to **7** but with a larger amplitude. The two helimers showed crossover at 233 and 263 nm. The positive CD spectra for **7** suggested the helix with right-handedness and hence *P* conformation, while **8**, with left-handedness, has *M* configuration.[5c] The CD amplitude both showed strong dependency on the concentration of **7** and **8** (data not shown).

As NCSi-gb has conformed specific binding to bulged DNA,[10] we wished to know if its analogue-compounds **7** and **8** also display the specific recognition and binding ability to bulged DNA. To gain insight into the chiral nature and the dynamics of bulged DNA binding to a drug, several bulge-containing oligonucleotides[5b,5e] shown in Figure 3 were selected as binding hosts for diastereomers **7** and **8**.

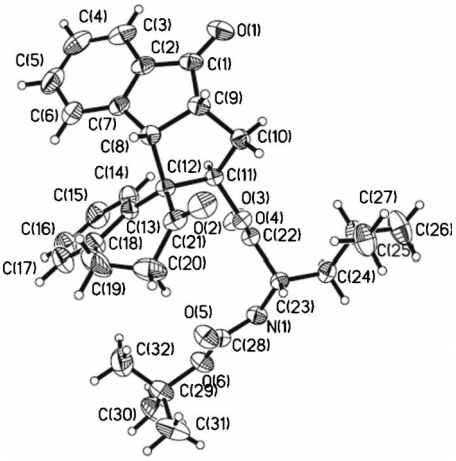


Figure 1. ORTEP of compound **5**.

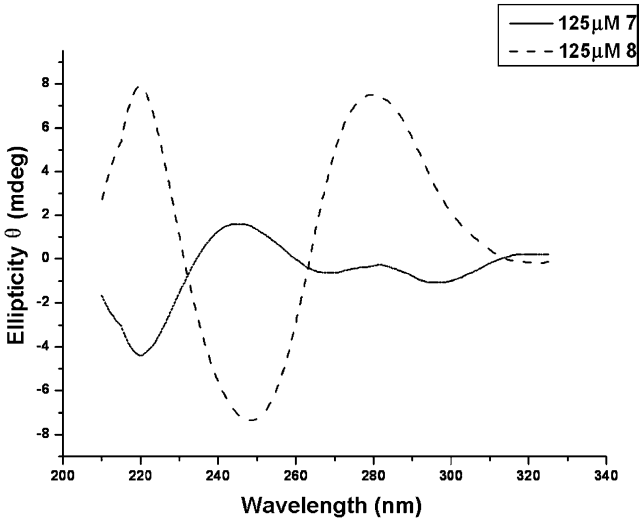


Figure 2. CD spectra of compounds **7** and **8**. The CD spectra were performed on a Jasco-715 spectropolarimeter, using quartz cylindrical cell of 1 mm path length. The cell compartment was continuously purged with dry N_2. Data were recorded at a bandwidth of 1.0 nm and measured at every 0.2 nm over of 210–325 nm at 20 °C in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) containing 1 mM NaCl.

1	HT3AT	5'-GTC CGA TGC GTG T 3'-CAG GCT ACG CAC T	T Tm=80.20 ℃
2	HT3AGT	5'-GTC CGA TGC GTG T 3'-CAG GCT ACG CAC T G	T Tm=70.25 ℃
3	HT3AGTT	5'-GTC CGA TGC GTG T 3'-CAG GCT ACG CAC T TG	T Tm=64.90 ℃
4	HT3AGCTT	5'-GTC CGA TGC GTG T 3'-CAG GCT ACG CAC T T C G	T Tm=63.30 ℃

Figure 3. Sequences, tentative secondary structures, and measured melting temperature (T_m) of the selected four DNA strands.

CD spectropolarimetry can be used to monitor the conformational transitions as the ligand–nucleic acid complex formed. Figure 4 shows the circular dichroism titration spectra of the complex of one-base bulge DNA (HT3AGT) with **7** (Fig. 4A) and **8** (Fig. 4B), respectively. The observed CD spectrum of the selected native DNA consists of a distinct positive band at 280 nm due to base stacking and a negative band at 250 nm due to helicity,[17,18] which is characteristic of DNA in right-handed B form. Both CD spectra of DNA with different concentrations of drug **7** (Fig. 4A) and **8** (Fig. 4B) consistently revealed an isoelliptic point at about 260 nm, indicating the formation of a drug–DNA complex. The changes in the CD spectra of DNA and its complex upon addition of increasing amounts of **7** showed a blueshift of the peak of the positive band from 250 to 246 nm (Fig. 4A). Figure 4B with **8** also shows a gradual blueshift of the peak of the negative band from 250 to 247 nm. The insets in Figures 4A and B show the single-wavelength titration curves obtained from the CD amplitudes at 250 nm as a function of molar concentrations of added **7** and **8** to the DNA, respectively. All four selected DNA strands displayed analogue CD characteristic (data not shown).

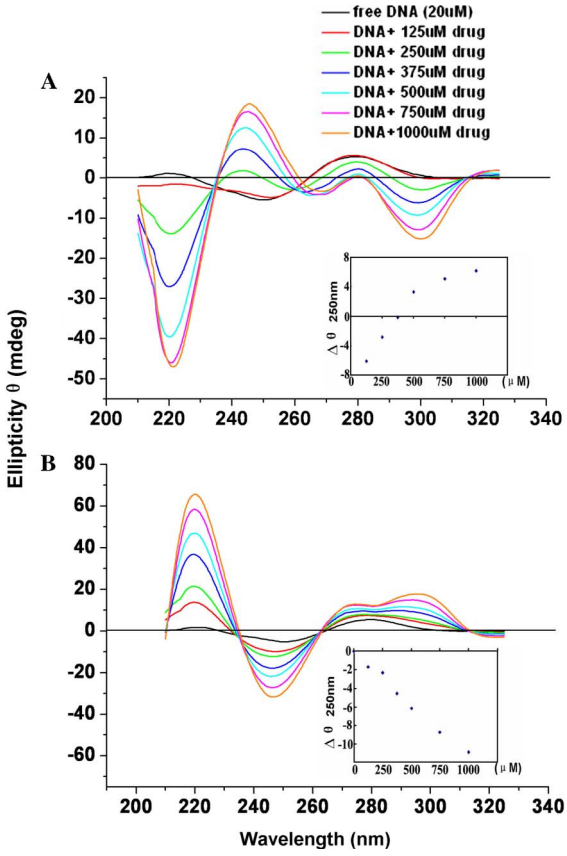


Figure 4. Circular dichroism titration spectra of complex of one-base bulge DNA (HT3AGT) with drugs **7** (A) and **8** (B). The concentration of drugs added and corresponding colored lines are indicated. CD spectra were recorded as Figure 3. All oligonucleotides (20 μM) were heated to 95 °C for 5 min and then cooled to room temperature slowly before use. The inset shows the single-wavelength titration curves obtained from the CD amplitudes at 250 nm as a function of molar concentration of added drug to the DNA.

In order to observe the conformational transitions of DNA directly and to eliminate interferences of drugs, the circular dichroism spectra of the native DNA and its altered DNA after subtracting the drug-alone spectrum from the complex are shown in Figure 5, by assuming that the conformation of the drug was not significantly altered since the molecular models of **7** and **8** were fairly rigid. For the oligomers with hairpin structure (HT3AT), there was no overall ellipticity change detected from the differential spectra of DNA (Figs. 5A and B). The binding of **7** or **8** to DNA might be through simple groove binding and/or electrostatic interaction which showed less or no perturbations on the base stacking and helicity bands,[19] ruling out the possibility of conformational change.

For the oligomers containing single (1- to 3-base) bulges, the spectrum of drug-subtracted DNA (dashed line) compared to that of the native DNA (solid line) had been significantly changed. When the one-base bulge oligomer (HT3AGT) interacted with compound **7**, the altered DNA spectrum showed a small increase in the positive band at 280 nm and a large decrease in the negative band at 246 nm (Fig. 5C), indicating an alteration in the DNA conformation because of an overall bending of the DNA backbone.[17] When HT3AGT interacted with compound **8**, the altered DNA spectrum (Fig. 5D) showed similar change to that of **7**, but with less magnitude.

The adding of compound **7** to the two-base bulge (HT3AGTT) caused the altered DNA (dashed line) spectrum (Fig. 5E) to change significantly. A redshift of the negative band at 250 nm and the newly appeared positive band at 242 nm indicated that the conformation of the bulged stem might change greatly after the binding of the drug. The compound **8** gave another interaction mode with HT3AGTT compared to **7**. The altered DNA (dashed line) spectrum (Fig. 5F) exhibited an increase of the positive band at 280 nm and a decrease of the negative band at 249 nm, and the negative band was redshifted by 5 nm. The wavelength of 280 nm of the spectrum due to π–π* transition was quite sensitive to the base pair of DNA[18,19] and might be influenced

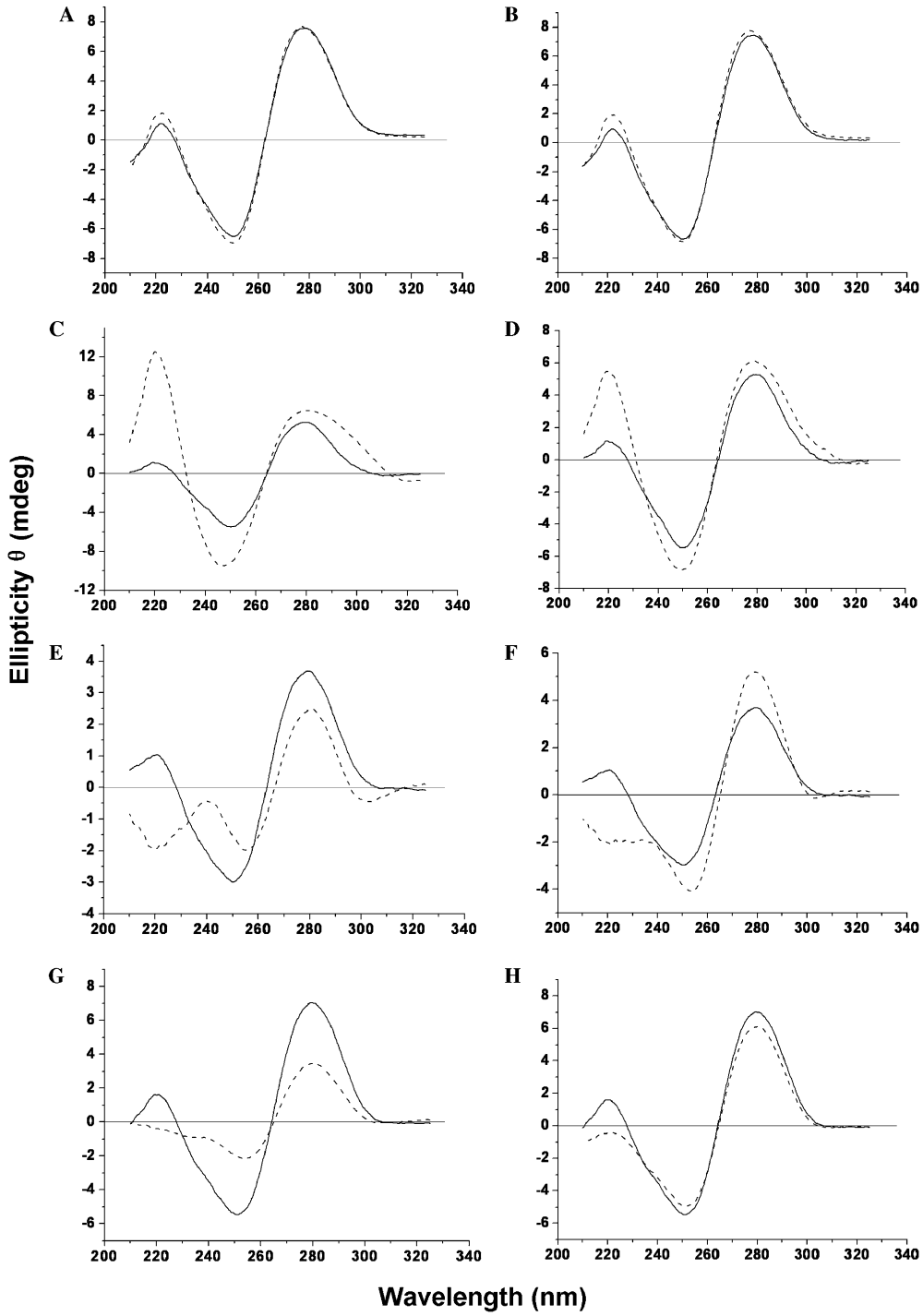


Figure 5. Circular dichroism spectra of DNAs (20 μM) alone (solid line) and drug–DNAs complex after subtraction of drug (250 μM) alone (dashed line). DNA and drug used are: (A) HT3AT + **7**; (B) HT3AT + **8**; (C) HT3AGT + **7**; (D) HT3AGT + **8**; (E) HT3AGTT + **7**; (F) HT3AGTT + **8**; (G) HT3AGCTT + **7**; (H) HT3AGCTT + **8**.

by the alterative stacking of base pairs. The changes of the negative band at 249 nm suggested a tightly binding complex was formed and as such forced a perturbation of the DNA backbone.[17,20]

HT3AGCTT with 3-base bulge displayed distinct effect when it interacted with compounds **7** and **8**, respectively. There was no overall ellipticity change detected from the altered DNA (dashed line) spectrum (Fig. 5) after the interaction between HT3AGCTT and **8**, indicating that the binding of **8** did not influence the overall conformation of DNA. On the contrary, greater positive and negative ellipticity changes were generated in the altered DNA (dashed lines) spectrum from the drug **7**–DNA complex in Figure 5G. A redshift of the negative band at 250 nm and the newly appeared positive band at 242 nm indicated that though the duplex structure of the oligomer was not obviously unwound, the conformation

of the bulged stem distorted heavily. The decreased long wavelength band was reminiscent of the spectra of dehydrated DNA in a non-standard B conformation.[17] The result implied that the binding pocket of the 3-base bulge DNA was large enough to permit the compound **7** to enter into and to squeeze water molecules buried in the base pairs out, turning into a more dehydrated form.

CD results indicated that both hydrophobic NCS-chrom mimics **7** and **8** displayed specific recognition and binding ability to bulged DNA by shifting the bulged distortion away from the original location. Our experiments followed an 'induced fit' mechanism[18] in the recognition event of the drug altering the conformation of bulged DNA. This highly cooperative 'induced fit' binding behavior appeared to enhance both hydrogen bonding interactions and van der Waals contacts between the ligand and atoms lining the walls of the grooves, which were generally energetically favorable and maximized through the structural accommodations providing the drug–DNA complex with a source of enhanced stability. Both of the synthesized compounds **7** and **8** were able to achieve the proper phasing of its aromatic subunits with the edges of the base pairs in the groove by altering its own inherent twist, thus intermediate to alter the conformations of the host-bulged DNA specifically.

Compounds **7** and **8** exhibited an obviously different behavior to the various bulged DNA hosts. Compound **7** was one benzene ring short as to NCSi-gb in spirocyclic system.[5c] It possessed the geometry for mimicking natural helical bases and formed a wedge by the two rigidly held ring systems.[21] Though compound **8** also formed a similar molecular wedge, the orientation and angle while it interacted with the DNA bulge might be quite a different from that of **7**, thus it induced quite different conformational change of bulged DNA. The geometric configuration at the attached site between the side chain and spiroalcohol is different in between natural metabolite NCS-gb and compounds **7** and **8**. These differences result in different binding conformations, which can also be seen in the solution NMR and CD studies.[20,21] The actual binding differences among DNA bulge-binding molecules, such as NCS-gb, DDI,[5a,5b] and compounds **7** and **8** (with relatively small side chain), need to be clarified in further NMR studies.[7,11]

The higher-ordered nucleic acid structures (RNA and DNA) such as bulges and hairpins are ubiquitous components that are often associated with important biological functions. A thorough understanding of the tertiary DNA recognition process of the mimic of NCS-chrom will allow further developments in the area of rational drug design targeting on the DNA bulge-related structure,[5,22] which might interfere with nucleotide expansions found in various disease states and also some important process in RNA virus infections.

Acknowledgments

We thank NSFC (20272029, 20432010), NSF of Tianjin (013616311), MOST (2003CB114403), and MOE of China for financial support. We also wish to thank I. H. Goldberg for the help in the manuscript preparation.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.11.084.

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14. Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (d, J = 7.1 Hz, 1H), 7.32–7.14 (m, 4H), 6.81 (t, J = 6.7 Hz, 1H), 6.21 (d, J = 7.1 Hz, 1H), 6.04 (d, J = 7.6 Hz, 1H), 5.41 (t, J = 7.5 Hz, 1H), 4.16 (d, J = 6.5 Hz, 1H), 3.89–3.80 (m, 1H), 3.42–3.12 (m, 3H), 2.95–2.91 (m, 1H), 2.77–2.65 (m, 3H), 1.25 (br s, 2H), 0.92 (d, J = 6.7 Hz, 1H), 0.84 (br s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.46, 207.19, 168.93, 152.27, 137.85, 137.48, 134.21, 133.73, 128.32, 128.23, 128.09, 127.71, 126.47, 123.66, 80.52, 62.08, 51.23, 49.35, 48.85, 40.07, 39.54, 30.70, 28.49, 24.20, 21.98, 21.55. MS (ESI, positive): Anal. Found: *m/e* 432 $(M+1)^+$; Calcd for $C_{27}H_{29}NO_4$ 431.
15. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 7.5 Hz, 1H), 7.33–7.16 (m, 4H), 6.84 (t, J = 6.6 Hz, 1H), 6.20 (d, J = 8.0 Hz, 1H), 6.10 (d, J = 7.4 Hz, 1H), 5.36–5.30

(m, 1H), 4.16–4.09 (m, 2H), 3.909–3.81 (m, 1H), 3.48–3.12 (m, 3H), 2.96–2.92 (m, 1H), 2.76–2.65 (m, 3H), 1.28–1.22 (m, 2H), 0.94–0.90 (m, 1H), 0.57 (br s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.05, 208.25, 169.57, 152.42, 137.98, 137.40, 134.47, 133.70, 128.47, 128.31, 128.13, 127.91, 126.43, 123.78, 80.87, 61.87, 51.64, 49.43, 48.67, 40.11, 39.34, 30.48, 28.63, 24.09, 21.86, 21.22. MS (ESI, positive): Anal. Found: *m/e* 432 $(M+1)^+$; Calcd for $C_{27}H_{29}NO_4$ 431.

16. CCDC 272414 contains the supplementary crystallographic data for compound **5**. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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