Enantioselective Synthesis of Clavepictine Analogues and Evaluation of Their Cytotoxic Activity

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Six analogues (labeled **27** to **32**) of a cytotoxic alkaloid isolated from the tunicate *Clavelina picta* were synthesized from an acyl oxazolidine. The absolute stereochemistry of the targeted analogues derived from (*R*)-phenyl glycinol and the relative stereochemistries of three of the four stereocentres present in the molecule were set up by stereocontrolled additions to transient iminium ions. The main features of this synthesis include (i) a high level of stereocontrol for all the steps involving the arrangement of relative stereochemistries, (ii) a divergent introduction of the side chain at the end of the synthesis, allowing the easy preparation of the different analogues, and (iii) an original step involving an intramolecular alkylation of an aminonitrile moiety that enabled the efficient construction of the quinolizidine core to take place. Together with the cytotoxic activities of the six analogues, those of three reference compounds (etoposide, adriamycin and irinothecan) were determined by means of the colorimetric MTT assay on four human-cancer cell lines. Compound **31** had a cytotoxic effect on the four human-cancer cell lines in dose ranges similar to etoposide and irinothecan. Compound **30** also had a significant cytotoxic effect on all four of the human-cancer cell lines under study, but these activities were weaker than those induced by compound **31**. Compound **29** had a significant cytotoxic effect on three of the four human-cancer cell lines, and compounds **27**, **28** and **32** had no cytotoxic effect (except compound **27** with respect to the A549 model at the highest dose) on the four human models under study.
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Introduction

Biologically active compounds from marine organisms have been the subject of intense research over recent decades,[1] and although their complicated and fascinating structures have provided many challenges for chemists, very few have been developed as viable new drugs. This situation is, of course, the result of their poor availability and costly synthesis, which is mainly reserved to academic research. As regards anticancer molecules, sponges and tunicates have provided particularly potent and fascinating new compounds, such as bryostatin-1[2] and dolastatin-10.[3] Of these, the former is currently undergoing Phase II clinical trials while the latter is claimed to be the most potent neoplastic substance known. In this field, Raub et al.[4] isolated new quinolizidinic alkaloids from the tunicate *Clavelina picta*. These compounds, clavepictine A (**1**) and B (**2**) (Figure 1), showed a potent cytotoxic effect in different cancer cells lines (vide supra). The total synthesis of these molecules was first accomplished by the Momose group in 1996, and required 32 steps.[5] Later, Ha and Cha[6] reported a synthesis in 27 steps. The length of these syntheses highlights the difficulty inherent in accessing these molecules and explains why no SAR related to these cytotoxic compounds has been mentioned so far in the literature. In this article we report a shorter (15 steps) and divergent synthesis of clavepictine analogues and the cytotoxic activity of these compounds with respect to four different cancer cells lines.

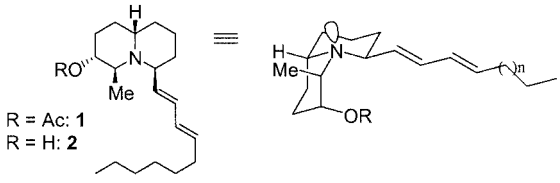

Figure 1. Natural clavepictines isolated from *Clavelina picta*

The targeted analogues were selected on the basis of the hypothesis that the cytotoxic activity of natural clavepictines might be due to their ability to generate aziridinium ions through the intramolecular displacement of the β-hydroxylic centre (activated as a leaving group in the physiological medium) by the nucleophilic tertiary amine. This intramolecular displacement is possible, however, only if the

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molecule changes its conformation from **2a** (favoured energetically)[4] to **2b** (Figure 2). Since aziridinium ions are known to be potent electrophiles, cytotoxic activity might result from the alkylating properties of these molecules. Although this hypothesis has no experimental support, it is interesting to notice that numerous alkaloids presenting a cytotoxic effect, such as indolizomycin (**3**),[7] (+)-allopumiliotoxin 267-A (**4**),[8] and (+)-micropine (**5**),[9] include in their structure the pattern necessary to generate aziridinium ions (Figure 3).

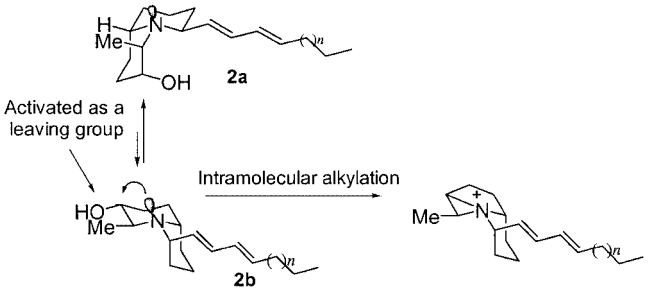


Figure 2. A putative mechanism involving generation of electrophilic aziridinium ions from clavepictines

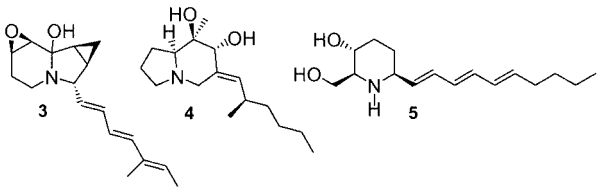


Figure 3. Some alkaloids showing the substructure necessary to generate aziridinium ions

On the basis of this hypothesis, clavepictine analogues with a *trans* ring junction and a variable side chain (R in Figure 4) were selected as target molecules with the aim of improving their alkylating properties. Indeed, the stable conformation of the quinolizidine ring in these isomers puts the tertiary amine lone pair of electrons in a good position to generate aziridinium ions through intramolecular substitution.

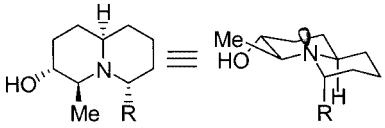
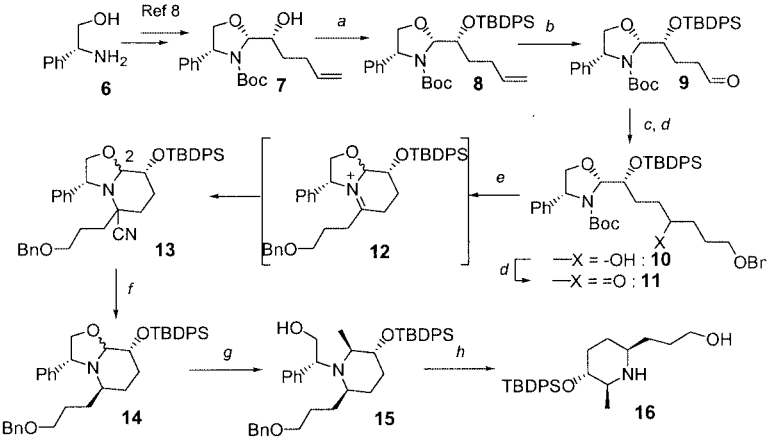


Figure 4. Selected target analogues with a *trans* ring junction

Chemistry

The synthesis begins with alcohol **7**, previously reported as an intermediate in the synthesis of (−)-desoxoprosopinine.[10] This alcohol is easily prepared in five steps from (*R*)-phenylglycinol (**6**) as the source of chirality, and is transformed into ketone **11** using the sequence in Scheme 1. The treatment of this ketone with trifluoroacetic acid induced *N*-Boc deprotection and intramolecular condensation, thus generating an iminium ion **12** that was trapped by a cyanide ion, to afford amino nitrile **13** as a 7:3 epimeric mixture at C-2. This epimerization, resulting from an equilibration of the amino ether moiety, is of no consequence for the outcome of the following steps. The reduction of this α-aminonitrile was effected by treating it with silver tetrafluoroborate to abstract a cyanide ion from the amino nitrile moiety, and this step was followed by reduction with zinc borohydride.[11] Thus, bicyclic oxazolidine **14** was obtained with total stereocontrol as an epimeric mixture at C-2. The treatment of **14** with methylmagnesium bromide then introduced the methyl substituent, which gave trisubstituted piperidine **15** in a totally stereoselective way. Finally, the hydrogenolysis of this compound in the presence of Pearlman's catalyst gave **16**.

Once we had multigram quantities of this piperidine at our disposal, we turned our attention to the construction of the quinolizidinic ring. For this purpose, the secondary amine in **16** was alkylated with bromoacetonitrile to give



Scheme 1. Reagents and conditions: (a) NaH, THF, then TBDPSCl, 55−60 °C, 92%. (b) OsO_4 (cat.), $NaIO_4$, THF/H_2O, room temperature, 91%. (c) $BnO(CH_2)_3MgBr$, Et_2O, room temperature, 98%. (d) $(COCl)_2$, DMSO, Et_3N, DCM, −50 °C, 94%. (e) TFA, DCE, room temperature then KCN/H_2O, 89%. (f) $AgBF_4$, THF, room temperature, then $Zn(BH_4)_2$, −78 °C, 85%. (g) MeMgBr, THF, room temperature, 89%. (h) H_2, $Pd(OH)_2$, EtOH, quant.

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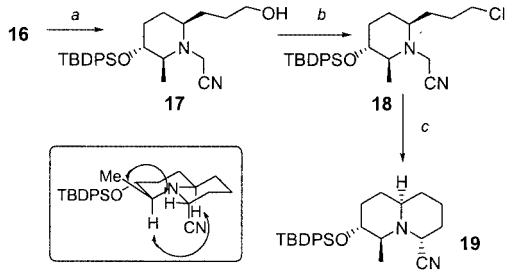
17, which was treated with thionyl chloride. The so-produced chloride **18** was then subjected to ring closure through the intramolecular alkylation of the metallated aminonitrile to afford quinolizidine **19** as a single isomer.[12] The structure of this compound was determined on the basis of NMR spectroscopic data (see the Exp. Sect.) and was confirmed by NOE experiments (Scheme 2).



Scheme 2. Reagents and conditions: (a) $BrCH_2CN$, K_2CO_3, CH_3CN, reflux, 93%. (b) $SOCl_2$, DCM, reflux. (c) LiHMDS, THF, −78 °C, 89% overall

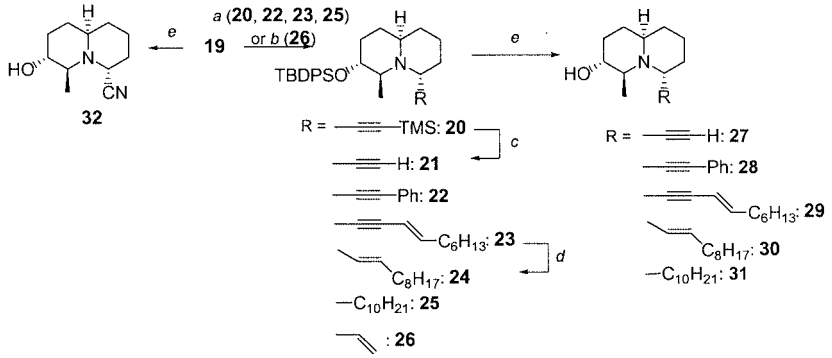
Amino nitrile **19** proved to be a valuable precursor in the one-step preparation of a diverse range of clavepictine analogues since side chains of various lengths and degrees of unsaturation can be introduced from this compound with good yields and high levels of stereocontrol. To this end, this aminonitrile was reacted with alkynyl or saturated Grignard reagents to give quinolizidines **20**, **22**, **23**, and **25** as single isomers. The introduction of an sp^2-hybridised Grignard reagent to afford vinylic quinolizidine **26** was also successful, provided that the amino nitrile was treated with silver tetrafluoroborate prior to its reaction with vinylmagnesium bromide.[13] The desilylation of alkyne **20** gave **21**, and the reduction of enyne **23** with sodium in liquid ammonia gave *E*-alkene **24** as a major product. The final deprotection of **19** and **21**−**25** using TBAF in THF gave hydroxy quinolizidines **27**−**32** (Scheme 3). We mention in passing that different strategies were developed with the aim to introduce an (*E*,*E*)-decadienyl side chain similar to the one present in natural clavepictines, but they met with no success.

The high levels of stereoselectivity attained in the transformations of **13** into **14** and **14** into **15**, and in the introduction of the side chain from **19**, deserve some comments. All these reactions involve intermediate iminium ions as reactive species, and the high diastereoselectivity can be explained by stereoelectronic effects.[14] With regard to the stereoselective reduction of **13** into **14**, Figure 5 shows the two possible half-chair conformers, **12a** and **12b**, of the iminium ion resulting from cyanide abstraction from aminonitrile **13**.[15] Axial attack of a hydride ion occurs on the top face of the iminium ion in conformer **12a** and on the bottom face in conformer **12b**. Although the preferred pathway is an attack on **12a** when the hydroxyl protecting group is a benzyl group [as evidenced in a previously reported synthesis of (−)-desoxoprosopinine][10] or when the substrate is devoid of a hydroxylated centre,[11] the bulkiness of the TBDPS protecting group in our substrate disfavours an axial attack on conformer **12a** because this hindered substituent would occupy an axial position in the corresponding transition state and would experience a severe steric interaction with the phenyl substituent on the oxazolidine ring. In our case, therefore, the preferred reaction pathway occurs as the result of an axial attack on conformer **12b**. This result shows that it is possible to control the sense of this reduction simply by using different protecting groups on the hydroxyl moiety (Figure 5).

Concerning the addition of the methyl substituent (transformation of **15** into **16**), the stereoselectivity can be explained once again by an axial attack on an intermediate iminium ion anti to the large TBDPS ether.[10] Finally, the stereoselective introduction of the Grignard reagents from amino nitrile **19** is rationalised also by a stereoelectronic effect: e.g., an axial attack on the intermediate iminium ion **33** (Figure 5).

Pharmacology

Five concentrations of each of the compounds under study were tested on four different human-cancer cell lines,



Scheme 3. Reagents and conditions: (a) RMgBr, THF, room temperature, quant. (**20**), 89% (**22**), 84% (**23**), 93% (**25**). (b) $AgBF_4$, THF, room temperature, then vinylmagnesium bromide, −78 to 0 °C, 80%. (c) K_2CO_3, MeOH, quant. (d) Na, liq. NH_3, THF, −78 °C, 51%. (e) TBAF, THF, 50 °C, 68% (**27**), 68%, (**28**), 77% (**29**), 65% (**30**), 76% (**31**), 84% (**32**)

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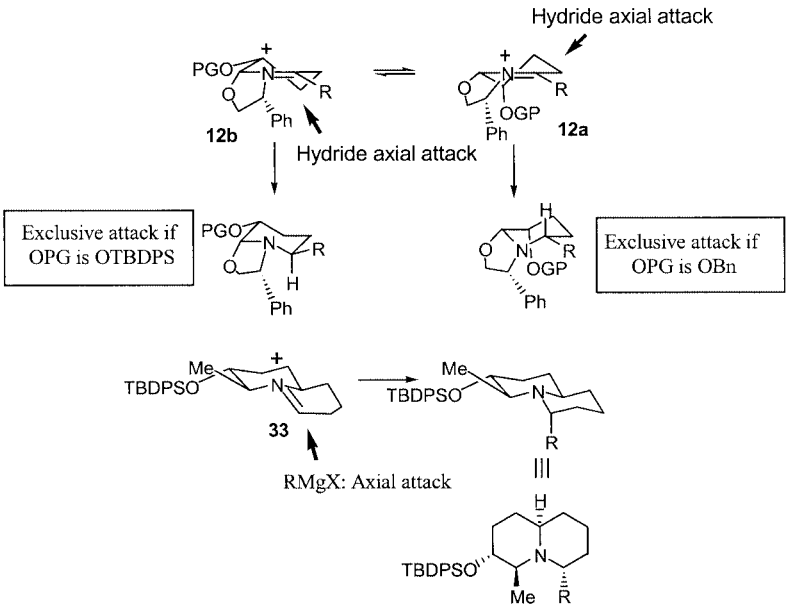

Figure 5. The stereoselectivity of the reduction of amino nitrile **13** and of the alkylation of amino nitrile **19** can be explained by steroelectronic effects that govern the attack of intermediate iminium ions

including various histopathological types (a glioblastoma, and colon, lung, and bladder cancers). Natural clavepictines A (**1**) and B (**2**) are not included for direct comparison in this study, because of their lack of availability, but these compounds were reported to present cytotoxic activities (IC_{50}) in the range of 1.8 to 8.5 μg/mL against murine leukemia and human solid-tumor cell lines (P-388, A-549, U-251, and SN12 K1).[4] We made use of the colourimetric MTT assay, which indirectly assesses the effect of potential anticancer compounds on the overall growth of adherent cell lines.[16,17] The percentages of tumor cells surviving in each experimental conditions were determined after four days of culture in the presence (or absence, i.e., control) of the drugs (see Table 1). The percentages of cells of each of the six cancer models present in the control were normalised arbitrarily at 100%. This normalisation enabled a clear analysis to be made in the case of the cytotoxic effects induced by a given compound. We used adriamycin, etoposide, and irinothecan, three cytotoxic reference compounds known as to be clinically active.

The data in Table 1 show that, when administered in dose ranges similar to those of etoposide and irinothecan (two of the three reference compounds), at least one (compound **31**) of the six compounds under study had a cytotoxic effect on four human-cancer cell lines. Indeed, while etoposide was more cytotoxic than **31** on the U373 glioblastoma, the colon HCT-15 and the J82 bladder-cancer models, **31** was more cytotoxic against these three cancer cell lines than irinothecan. As for the A549 non-small-cell lung-cancer model — the fourth model under investigation — compound **31** displayed a level of cytotoxicity similar to that displayed by etoposide. Furthermore, at both 10^{-5} and $5{\cdot}10^{-6}$ M, compound **31** was more cytotoxic than irinothecan against the U373, the HCT-15, the A549, and the J82 human-cancer cell lines. We emphasize that **31** differs from the other compounds under study in that its side chain is long and fully saturated. Of the remaining compounds under study, only compound **30** had any significant cytotoxic effect on all four of the human-cancer cell lines under study. The cytotoxic effects exhibited by **30**, however, were systematically lower than those exhibited by **31**. In terms of chemical structures, compound **30** differs from compound **31** by the presence of an unsaturated side chain that restricts its conformational mobility. Compound **29** had a significant cytotoxic effect on three of the four human-cancer cell lines investigated here. We note that the higher degree of unsaturation on the side chain in **29**, as compared to **31** and **30**, lowers its conformational mobility to a greater extent. The fourth human-cell line upon which **29** had no significant cytotoxic effect was the A549 non-small-cell lung-cancer model. In fact, this human-cancer cell line was also the one that exhibited the highest level of chemoresistance to the reference compounds, etoposide and irinothecan. In contrast, **31** had a dramatic cytotoxic effect on this aggressive human-cancer model.

Finally, compounds **27**, **28**, and **32** had no cytotoxic effect (except **27** at the highest dose in the case of the A549 model) on the four human models under study. These three compounds differ chemically from **29**, **30**, and **31** by their considerably shorter side chains.

Each experimental condition was tested in sextuplicate. The data are presented as means ±SEM values. The optical density values given by the colorimetric MTT assay (see Materials and Methods) in the control have been normalized at 100%. Thus, for example, a value of 41% in Table 1 indicates that 41% of the treated cells survived in this experimental condition relative to the corresponding control value. The stars represent the p levels of statistical significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (Mann−Whitney U test).

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Table 1. Characterization of the cytotoxic activities of clavepictine analogues on four human-cancer cell lines

Cell line	Drugs	Drug concentration [M]				
		10^{-5}	$5\cdot10^{-6}$	10^{-6}	$5\cdot10^{-7}$	10^{-7}
U373	etoposide	54±4***	64±2***	80±3**	87±3*	105±3
−	adriamycin	20±2***	21±2***	34±1***	39±1***	57±3**
−	irinothecan	64±2***	66±2***	109±3	106±4	106±3
−	**27**	102±2	102±4	100±4	102±4	100±3
−	**28**	93±2	96±2	98±3	98±3	100±4
−	**29**	40±1***	90±1**	95±3	96±2	105±3
−	**30**	15±1***	52±2***	100±4	98±2	103±4
−	**31**	6±2***	15±2***	108±3	106±4	106±4
−	**32**	97±2	97±4	93±5	95±3	102±3
HCT-15	etoposide	35±1***	46±2***	70±2***	77±2***	98±3
−	adriamycin	21±2***	24±1***	34±2***	41±3***	63±3***
−	irinothecan	77±2***	87±3*	102±3	99±3	102±5
−	**27**	100±2	101±3	103±3	101±2	107±3
−	**28**	102±3	106±3	106±4	101±2	106±3
−	**29**	46±2***	81±2***	104±4	107±3	104±4
−	**30**	48±3***	83±1***	102±3	102±3	102±4
−	**31**	10±1***	23±1***	103±3	99±4	102±4
−	**32**	100±3	97±3	104±3	100±2	104±3
A549	etoposide	71±4***	73±2***	93±3	95±2	103±2
−	adriamycin	10±1***	10±1***	28±2***	44±1***	65±3***
−	irinothecan	78±4**	93±3	102±3	103±3	102±5
−	**27**	87±3*	93±3	98±4	98±4	98±5
−	**28**	102±4	98±4	92±4	95±5	94±6
−	**29**	102±4	106±3	98±3	101±4	102±4
−	**30**	22±1***	105±3	96±4	98±4	98±4
−	**31**	9±1***	23±1***	91±3	99±4	99±3
−	**32**	96±3	97±3	95±3	93±4	96±4
J82	etoposide	46±3***	57±3***	78±4**	88±3*	109±3
−	adriamycin	3±1***	2±1***	29±1***	41±4***	62±2***
−	irinothecan	81±2***	92±3	100±4	101±3	107±3
−	**27**	104±4	105±3	106±3	103±4	99±5
−	**28**	101±3	98±3	101±3	102±4	102±4
−	**29**	9±1***	51±5***	105±4	104±2	106±5
−	**30**	13±1***	55±4***	104±3	105±4	107±4
−	**31**	4±2***	14±1***	107±3	105±4	106±3
−	**32**	100±3	96±4	103±4	101±4	99±3

Conclusions

A short and efficient asymmetric synthesis of clavepictine B analogues has been devised. This synthesis permitted the divergent preparation of six analogues differing in the nature of their side chains on the quinolizidine skeleton. The evaluation of the cytotoxic activity of these compounds was carried out in relation to four different human-cancer cell lines, and the results enable two important conclusions to be drawn regarding the structure/activity relationships of this type of compound. Firstly, the length and conformational mobility of the side chain on the quinolizidine heterocycle seems to be an important parameter for a high level of cytotoxic effectiveness. In addition, we note that the presence of unsaturation in the side chain alters the activity, and note that a diene moiety is present in the side chain of natural clavepictines. Secondly, the relative configuration (*trans* ring junction) in these analogues differs in comparison with the natural clavepictines. This modification at the level of the relative stereochemistry, a modification that affects the overall conformation of the quinolizidine heterocycle, does not seem to alter the cytotoxic activity. These first insights into the SAR of these molecules will now help us to select other target analogues in order to optimize their activities.

Experimental Section

Chemistry

General Remarks: ^{1}H and ^{13}C spectra ($CDCl_3$ solution) were recorded on a Bruker ARX 250 spectrometer at 250 and 62.9 MHz respectively; chemical shifts are reported in ppm relative to TMS. Optical rotations were determined with a Perkin−Elmer 141 instrument. All the reactions were carried out under argon. Column chromatography was performed on a silica gel 230−400 mesh by using various mixtures of diethyl ether (Et_2O), ethyl acetate (EtOAc), and petroleum ether (PE). The TLC experiments were run on Merck Kieselgel $60F_{254}$ plates. The melting points are uncorrected. THF and ether were distilled from sodium/benzophenone ketyl. Dichloromethane was distilled from calcium hydride. The mention of a "usual workup" means: (i) decanting the organic layer, (ii) extracting the aqueous layer with ether, (iii) drying the combined organic phases with $MgSO_4$, (iv) and evaporating solvent under reduced pressure. The composition of the stereoisomeric mixtures was determined by NMR spectroscopic analysis on crude products before any purification.

[2*R*,4*R*,2(1*R*)]-3-*tert*-Butoxycarbonyl-2-(1-*tert*-butyldiphenylsilyloxypent-4-enyl)-4-phenyl-1,3-oxazolidine (8): Sodium hydride (60% wt. dispersion in mineral oil, 1.2 g, 30.0 mmol) was added portionwise to a solution of **7** (5.0 g, 15.0 mmol) in dry THF (800 mL). The mixture was stirred for 1 h prior to the addition of *tert*-butyldiphenylsilyl chloride (12.0 mL, 46.1 mmol). After 12 h of stirring between 55 and 60 °C, the mixture was hydrolysed by the addition of saturated aqueous NH_4Cl. Most of the THF was then evaporated under reduced pressure and the residue was partitioned between ether and water. The usual workup gave a residue that was chromatographed (PE, then EtOAc/PE, 3:97) to give **8** as a clear oil. R_f = 0.66 (E/PE, 2:8). $[\alpha]_D^{20}$ = −15 (c = 0.7, $CHCl_3$). IR: $\tilde{\nu}$ = 3071, 2924, 1693 cm^{-1}. ^{1}H NMR: δ = 0.97 (s, 9 H, *t*Bu), 1.19 (s, 9 H, *t*Bu), 1.47 (br. q, J = 7.5 Hz, 2 H, CH_2), 1.71−1.93 (m, 2 H), 4.04−4.15 (m, 3 H), 4.56−4.66 (m, 2 H, CH=C*H*$_2$), 4.85 (t, J = 6.2 Hz, 1 H, C*H*Ph), 5.16 (d, J = 3.2 Hz, 1 H, NC*H*O), 5.34 (ddt, J = 17.0, 10.5, 6.5 Hz, 1 H, C*H*=CH$_2$), 7.12−7.30 (m, 11 H, Ar), 7.62−7.66 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 19.5 (Cq), 27.1, 28.3 (CH_3), 29.6, 31.1 (CH_2), 61.0 (CH), 72.5 (CH_2), 73.8 (CH), 80.7 (Cq), 92.1 (CH), 114.2 (CH_2), 127.0, 127.3, 127.5, 127.6, 128.3, 129.6 (CHAr), 134.2 (Cq), 136.1, 136.2, 138.5 (*C*H=CH$_2$), 140.5 (Cq), 154.8 (C=O) ppm. $C_{35}H_{45}NO_4Si$ (571.8): calcd. C 73.51, H 7.93, N 2.45; found C 73.39, H 8.09, N 2.40.

[2*R*,4*R*,2(1*R*)]-3-*tert*-Butoxycarbonyl-2-(1-*tert*-butyldiphenylsilyloxy-4-oxobutyl)-4-phenyl-1,3-oxazolidine (9): A solution of osmium tetroxide in water (4% wt. solution, 3.7 mL, 0.58 mmol) was added to a solution of **8** (6.6 g, 11.5 mmol) in a mixture of THF/water (1:1, 200 mL) cooled to 0 °C. Sodium periodate (12.3 g, 57.5 mmol) was added after 15 min and the suspension is stirred at room temperature for 4 h. The mixture was then treated by a 10% wt. aqueous solution of sodium thiosulfate (200 mL) and extracted with ether. The organic phase was washed with 10% wt. aqueous solution of sodium thiosulfate (200 mL), brine (200 mL), and dried with $MgSO_4$. Concentration under reduced pressure gave a residue that was chromatographed (EtOAc/PE, 2:8) to give **9** as an oil that

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crystallized on standing (6 g, 91%). M.p. 29 °C; R_f = 0.43 (E/PE, 2:8). $[\alpha]_D^{20}$ = −12.5 (*c* = 0.7, $CHCl_3$). IR: $\tilde{\nu}$ = 3071, 2932, 2859, 2720, 2252, 1716 cm^{-1}. ^1H NMR: δ = 0.98 (s, 9 H, *t*Bu), 1.21 (s, 9 H, *t*Bu), 1.58−1.76 (m, 2 H, CH_2), 2.17 (td, *J* = 8.0, 1.5 Hz, 2 H, CH_2), 4.10 (d, *J* = 6.2 Hz, 2 H, CH_2), 4.17 (br. q, *J* = 4.5 Hz, 1 H, CHOSi), 4.89 (t, *J* = 6.2 Hz, 1 H, CHPh), 5.15 (d, *J* = 4.5 Hz, 1 H, NCHO), 7.15−7.33 (m, 11 H, Ar), 7.60−7.64 (m, 4 H, Ar), 9.20 (t, *J* = 1.5 Hz, 1 H, CHO) ppm. ^{13}C NMR: δ = 19.5 (Cq), 24.0 (CH_2), 27.1, 28.2 (CH_3), 39.8 (CH_2), 60.8 (CH), 72.5 (CH_2), 73.1 (CH), 81.0 (Cq), 92.0 (CH), 126.9, 127.3, 127.6, 127.8, 128.4, 129.8 (CHAr), 133.8, 133.9 (Cq), 136.0, 136.1 (CHAr), 140.2 (Cq), 154.8 (C=O), 202.1 (CH=O) ppm. $C_{34}H_{43}NO_5Si$ (573.8): calcd. C 71.17, H 7.55, N 2.44; found C 71.03, H 7.97, N 2.38.

[2*R*,4*R*,2(1*R*,4*RS*)]-2-(7-Benzyloxy-1-*tert*-butyldiphenylsilyloxy-4-hydroxyheptyl)-3-*tert*-butoxycarbonyl-4-phenyl-1,3-oxazolidine (10): A few drops of 1-benzyloxy-3-bromopropane were added to a suspension of magnesium (1.8 g, 74.1 mmol) in dry ether (5 mL). As soon as the reaction was initiated, a solution of 1-benzyloxy-3-bromopropane (9.1 g, 39.7 mmol) in ether (85 mL) was added dropwise over 2 h. After stirring for an additional 1 h, the Grignard solution was cooled to 0 °C and the aldehyde **9** (5.1 g, 8.9 mmol) was added dropwise in ether (85 mL). After 1 h at 0 °C, hydrolysis with an saturated aqueous solution of NH_4Cl (20 mL) was followed by the usual workup to yield a residue that was chromatographed (EtOAc/PE, 2:8, then gradient of an extra 10% EtOAc per liter of eluting solvent). Compound **10** was obtained as a clear oil (6.3 g, 98%) and was shown to exist as approximately a 1:1 ratio of stereoisomers at the newly created stereocentre. R_f = 0.23 and 0.34 (E/PE, 2:8). IR: $\tilde{\nu}$ = 3466, 3069, 2927, 1683 cm^{-1}. ^1H NMR (mixture of isomers): 0.95 (s, 9 H, *t*Bu), 1.19 (s, 9 H, *t*Bu), 1.13−1.57 (m, 8 H, 4 × CH_2), 1.85 (br. s, 1 H, OH), 2.99−3.13 (m, 1 H, CHOH), 3.31 (t, *J* = 6.1 Hz, 2 H, CH_2OBn), 4.00−4.07 (m, 1 H, CHOSi), 4.15 (dd, *J* = 10.2, 6.5 Hz, 2 H, NCHCH_2O), 4.38 (s, 2 H, OCH_2Ph), 4.87 (t, *J* = 6.5 Hz, 1 H, NCHPh), 5.18−5.25 (m, 1 H, NCHO), 7.13−7.21 (m, 16 H, Ar), 7.61−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 19.4 (Cq), 25.9, 26.1 (CH_2), 27.1 (CH_3), 27.4 (CH_2), 28.2 (CH_3), 32.6, 33.3, 33.8, 34.3 (CH_2), 61.1 (CH), 70.5 (CH_2), 70.9 (CH), 71.5, 72.9 (CH_2), 74.4 (CH), 80.9 (Cq), 91.8 and 91.9 (CH), 126.9, 127.3, 127.6, 127.8, 128.3, 128.4, 129.7 (CHAr), 133.9, 134.1, 134.3 (Cq), 136.1 (CHAr), 138.5, 140.2, 140.4 (Cq), 155.1 and 155.3 (C=O) ppm. $C_{44}H_{57}NO_6Si$ (724.0): calcd. C 72.99, H 7.94, N 1.93; found C 72.85, H 8.07, N 1.86.

[2*R*,4*R*,2(1*R*)]-2-(7-Benzyloxy-1-*tert*-butyldiphenylsilyloxy-4-oxoheptyl)-3-*tert*-butoxycarbonyl-4-phenyl-1,3-oxazolidine (11): Dry DMSO (2.4 mL, 33.5 mmol) was added dropwise at −50 °C to a solution of oxalyl chloride (1.5 mL, 17.2 mmol) in dry dichloromethane (25 mL). After 5 min of stirring, a solution of **10** (6.5 g, 9.0 mmol) in dichloromethane (50 mL) was added and the mixture was stirred at −50 °C for 40 min before triethylamine (7.9 mL, 56.8 mmol) was added dropwise. The mixture was then warmed to 0 °C over 2 h, and treated with water (50 mL). After the usual workup, flash chromatography yielded **11** as a clear oil (6.1 g, 94%). R_f = 0.32 (E/PE, 3:7). $[\alpha]_D^{20}$ = −17 (*c* = 0.6, $CHCl_3$). IR: $\tilde{\nu}$ = 3069, 3032, 2930, 1700 cm^{-1}. ^1H NMR: δ = 0.97 (s, 9 H, *t*Bu), 1.21 (s, 9 H, *t*Bu), 1.55−1.72 (m, 4 H, 2 × CH_2), 2.02−2.19(m, 4 H, 2 × CH_2), 3.27 (t, *J* = 6.2 Hz, 2 H, CH_2OBn), 4.03−4.17 (m, 3 H, CHOSi and NCHCH_2O), 4.35 (s, 2 H, OCH_2Ph), 4.87 (t, *J* = 6.5 Hz, 1 H, NCHPh), 5.15 (d, *J* = 3.0 Hz, 1 H, NCHO), 7.07−7.33 (m, 16 H, Ar), 7.60−7.63 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 19.5 (Cq), 23.8, 25.7 (CH_2), 27.1, 28.2 (CH_3), 38.6, 38.9 (CH_2), 60.9 (CH), 69.4 (CH_2), 70.9 (CH), 72.5, 72.8 (CH_2), 73.4 (CH), 80.9 (Cq), 92.1 (CH), 126.9, 127.3, 127.6, 127.7, 128.4, 129.5 (CHAr), 134.1(Cq), 136.0, 136.1 (CHAr), 138.5, 140.3 (Cq), 154.8, 209.9 (C=O) ppm. $C_{44}H_{55}NO_6Si$ (722.0): calcd. C 73.20, H 7.68, N 1.94; found C 73.05, H 7.69, N 1.93.

(3*R*,8*R*,9*RS*)-5-(Benzyloxypropyl)-8-*tert*-butyldimethylsilyloxy-3-phenyloxazolo[3,2-*a*]piperidine-5-carbonitrile (13): Trifluoroacetic acid (8.6 mL, 112 mmol) was added rapidly at 0 °C to a solution of **11** (5.4 g, 7.48 mmol) in 1,2-dichloroethane (120 mL). After 3 h of stirring at room temperature a solution of potassium cyanide (8.6 g, 112 mmol) in water (100 mL) was added. The emulsion was vigorously stirred for 40 min (*Caution: this step must be conducted under an efficient hood and the ensuing HCN must be trapped*), and treated with a saturated aqueous solution of $NaHCO_3$ (100 mL). The usual workup (*Caution: use an HCN trap on the rotary evaporator*) gave a residue that was chromatographed (EtOAc/PE, 1:9) to give **13** as a clear oil (4.2 g, 89%). This compound was obtained as a 63:37 epimeric mixture at C-9. Major isomer: R_f = 0.57 (E/PE, 4:6). ^1H NMR: δ = 1.01 (s, 9 H, *t*Bu), 1.45−1.60 (m, 4 H, 2 × CH_2), 1.75−2.00 (m, 4 H, 2 × CH_2), 3.27−3.47 (m, 3 H, CH_2 and OCHSi), 3.91 (dd, *J* = 7.6, 0.6 Hz, 1 H, NCHCHHO), 4.00 (dd, *J* = 7.6, 6.0 Hz, 1 H, NCHCHHO), 4.26 (dd, *J* = 6.0, 0.6 Hz, 1 H, NCHCHHO), 4.38 (s, 2 H, OCH_2Ph), 4.61 (d, *J* = 7.2 Hz, 1 H, NCHO), 7.16−7.31 (m, 16 H, Ar), 7.62−7.66 (m, 2 H, Ar), 7.71−7.75 (m, 2 H, Ar) ppm. ^{13}C NMR: δ = 19.4 (Cq), 23.8 (CH_2), 27.1 (CH_3), 29.3, 33.9, 34.4 (CH_2), 56.5 (Cq), 60.4 (CH), 69.4, 72.6 (CH_2), 72.9 (CH), 73.0 (CH_2), 91.7 (CH), 118.6 (CN), 127.5, 127.6, 127.7, 127.8, 128.4, 128.5, 128.6, 129.1, 129.6 (CHAr), 133.8, 134.7 (Cq), 135.7, 136.1 (CHAr), 138.1, 138.3 (Cq) ppm. Minor isomer: R_f = 0.65 (E/PE, 4:6). ^1H NMR: δ = 1.01 (s, 9 H, *t*Bu), 1.40−1.83 (m, 6 H, 3 × CH_2), 1.96 (td, *J* = 13.5, 2.1 Hz, 1 H, CNCCHH), 2.27 (td, *J* = 13.5, 2.1 Hz, 1 H, CNCHH), 3.27−3.32 (m, 3 H, 1.5 × CH_2O), 4.10 (s, 1 H, NCHO), 4.16 (s, 1 H, OCHSi), 4.30 (s, 2 H, OCH_2Ph), 4.45 (t, *J* = 8.0 Hz, 1 H, NCHCHHO), 4.59 (dd, *J* = 8.0, 4.6 Hz, 1 H, NCHCH_2O), 7.13−7.27 (m, 16 H, Ar), 7.50−7.54 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 14.2 (Cq), 23.9, 24.5 (CH_2), 27.0 (CH_3), 27.2, 37.2 (CH_2), 58.9 (Cq), 64.0, 65.8 (CH), 69.5, 70.9 (CH_2), 72.7 (CH_2), 92.2 (CH), 122.6 (CN), 125.9, 127.1, 127.3, 127.5, 127.7, 127.8, 128.4, 128.5, 128.7, 129.8, 130.0 (CHAr), 133.2, 133.4 (Cq), 135.7, 135.8 (CHAr), 142.4 (Cq) ppm. IR (mixture of isomers): 3069, 2858, 2250, 1960, 1894, 1738 cm^{-1}. $C_{40}H_{46}N_2O_3Si$ (630.9): calcd. C 76.15, H 7.35, N 4.44; found C 76.12, H 7.46, N 4.32.

(3*R*,5*R*,8*R*,9*RS*)-5-(Benzyloxypropyl)-8-*tert*-butyldimethylsilyloxy-3-phenyloxazolo[3,2-*a*]piperidine (14): Silver tetrafluoroborate (1.1 g, 5.65 mmol) was added to a THF (50 mL) solution of **13** (2.4 g, 3.80 mmol). The mixture was stirred for 15 min, during which time a white precipitate was formed that turned to grey-black. The suspension was then cooled to −78 °C and a solution of zinc borohydride was added dropwise (0.5 M solution in ether, 7.6 mL, 3.80 mmol). Stirring was maintained for 1.5 h at below −70 °C and the reaction medium was then treated by water (50 mL). Filtration over a pad of Celite rinsed with ether was followed by the usual workup. The residue was chromatographed (EtOAc/PE, 1:9) to give **14** as a clear oil (1.95 g, 85%). Since this compound is obtained as a 85:15 epimeric mixture at C-9, only the major isomer is described below. R_f = 0.60 and 0.65 (E/PE, 4:6). ^1H NMR: δ = 0.90−1.63 (m, 8 H, 4 × CH_2), 1.01 (s, 9 H, *t*Bu), 2.16−2.27 (m, 1 H, CHN), 3.15 (br. oct, *J* = 3.0 Hz, 2 H, CH_2OBn), 3.46−3.55 (m, 1 H, CHOSi), 1.40−1.83 (m, 6 H, 3 × CH_2), 1.96 (td, *J* = 13.5, 2.1 Hz, 1 H, CNCCHH), 2.27 (td, *J* = 13.5, 2.1 Hz, 1 H, CNCHH), 3.27−3.32 (m, 3 H, 1.5 × CH_2O), 4.10 (s, 1 H, NCHO), 4.16 (s, 1 H, OCHSi), 4.30 (s, 2 H, OCH_2Ph), 3.82 (dd, *J* = 8.0, 2.2 Hz, 1 H, NCHCHHO), 4.05 (dd, *J* = 8.0,

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6.7 Hz, 1 H, NCHCH*H*O), 4.25 (dd, *J* = 6.7, 2.2 Hz, 1 H, NC*H*CHHO), 4.29 (s, 2 H, OCH_2Ph), 4.30 (d, *J* = 5.0 Hz, 1 H, NCHO), 7.15−7.29 (m, 16 H, Ar), 7.62−7.65 (m, 2 H, Ar), 7.71−7.74 (m, 2 H, Ar) ppm. ^{13}C NMR: δ = 19.5 (Cq), 25.8 (CH_2), 27.2 (CH_3), 28.8, 30.2, 31.9 (CH_2), 54.5 (Cq), 60.9 (CH), 70.4 (CH_2), 72.8 (CH and CH_2), 73.8 (CH_2) 95.0 (CH), 127.4, 127.5, 127.6, 128.1, 128.4, 128.9, 129.5 (CHAr), 134.2, 135.2 (Cq), 136.0, 136.3 (CHAr), 138.7, 141.0 (Cq) ppm. IR (mixture of isomers): 3069, 3030, 2933, 2856, 1739, 1241, 1113 cm^{-1}.

[2*S*,3*R*,6*R*,1(1*R*)]-6-(3-Benzyloxypropyl)-3-*tert*-butyldimethylsilyloxy-2-methyl-1-(1-phenyl-2-hydroxyethyl)piperidine (15): A solution of methylmagnesium bromide (3 M solution in ether, 7.0 mL, 21.0 mmol) was added dropwise to a solution of **14** (1.45 g, 2.39 mmol) in THF (85 mL) cooled to −10 °C. The mixture was stirred at room temperature for 48 h and then hydrolysed by a saturated aqueous solution of NH_4Cl (50 mL). The usual workup followed by flash chromatography (EtOAc/PE, 1:9) gave piperidine **15** as a clear oil (1.33 g, 89%). R_f = 0.37 (E/PE, 3:7). $[\alpha]_D^{20}$ = −17 (*c* = 1.0, $CHCl_3$). IR: ṽ = 3422, 3069, 3029, 2932, 2857, 1739 cm^{-1}. ^{1}H NMR: δ = 0.63−1.27 (m, 6 H, 3 × CH_2), 0.85 (d, *J* = 7.2 Hz, 3 H, Me), 1.05 (s, 9 H), 1.40−1.54 (m, 1 H, NCHC*H*HCH$_2$CHOSi), 1.95−2.09 (m, 1 H, NCHCH*H*CH$_2$CHOSi), 2.70−2.77 (m, 1 H, NC*H*CH$_2$), 2.97 (t, *J* = 6.6 Hz, 2 H, CH_2OBn), 3.14 (qd, *J* = 7.2, 1.6 Hz, 1 H, NC*H*CH$_3$), 3.34 (br. s, 1 H, OH), 3.53−3.62 (m, 2 H, CH*H*OH and C*H*OSi), 3.77−3.85 (m, 2 H, NC*H*Ph and C*H*HOH) 4.30 (s, 2 H, OCH_2Ph), 7.14−7.34 (m, 16 H, Ar), 7.61−7.65 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 17.8 (CH_3), 18.2 (Cq), 20.8, 21.1 (CH_2), 26.1 (CH_3), 27.5, 27.8 (CH_2), 50.3, 59.3 (CH), 61.8 (CH_2), 66.3 (CH), 69.3 (CH_2), 70.8 (CH), 71.5 (CH_2), 126.5, 126.6, 126.7, 127.3, 129.7 (CHAr), 134.1, 134.3 (Cq), 136.0, 136.3 (CHAr), 138.7, 141.2 (Cq) ppm. $C_{40}H_{51}NO_3Si$ (621.9): calcd. C 77.25, H 8.27, N 2.25; found C 77.20, H 8.27, N 2.18.

(2*S*,3*R*,6*R*)-3-*tert*-Butyldimethylsilyloxy-6-(3-hydroxypropyl)-2-methylpiperidine (16): Palladium hydroxide (20% wt. on charcoal, 6 g) was suspended in a solution of **15** (2.9 g, 4.29 mmol) in ethanol (180 mL). The mixture was vigorously stirred under a hydrogen atmosphere for 96 h and then filtered through Celite, and rinsed with hot ethanol. Concentration under reduced pressure followed by chromatography (Et_2O/EtOH/40% NH_4OH, 95:3:2) gave **16** as a white solid (1.75 g, quant. yield). M.p. 97.5 °C, R_f = 0.44 (Et_2O/EtOH/40% NH_4OH, 95:3:2). $[\alpha]_D^{20}$ = −15 (*c* = 1.2, $CHCl_3$). IR: ṽ = 3336, 3274, 3070, 1112 cm^{-1}. ^{1}H NMR: δ = 0.93−1.72 (m, 8 H, 4 × CH_2), 1.04 (s, 9 H), 1.14 (d, *J* = 6.2 Hz, 3 H, Me), 2.44−2.54 (m, 1 H, NC*H*CH$_2$CH$_2$CHOSi), 2.66 (qd, *J* = 6.2, 5.0 Hz, 1 H, NC*H*CH$_3$), 3.12−3.22 (m, 1 H, C*H*OSi), 3.43−3.63 (m, 2 H, CH_2OBn), 7.43−7.65 (m, 6 H, Ar), 7.32−7.65 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 19.5 (Cq), 19.6 (CH_3), 27.1 (CH_3), 30.6, 32.7, 34.4, 35.4 (CH_2), 56.2, 58.2 (CH), 62.8 (CH_2), 76.3 (CH), 127.5, 127.7, 129.6, 129.7 (CHAr), 133.9, 134.8 (Cq), 136.0 (CHAr) ppm. $C_{25}H_{37}NO_2Si$ (411.6): calcd. C 72.94, H 9.06, N 3.40; found C 72.75, H 9.24, N 3.46.

(2*S*,3*R*,6*R*)-3-*tert*-Butyldimethylsilyloxy-1-cyanomethyl-6-(3-hydroxypropyl)-2-methylpiperidine (17): Potassium carbonate (160 mg, 1.16 mmol) and bromoacetonitrile (0.20 mL, 2.87 mmol) were added to a solution of **16** (398 mg, 0.97 mmol) in acetonitrile (10 mL). The mixture was heated under reflux for 12 h and then the solvent was evaporated under reduced pressure. The residue was taken up into water and ether and the usual workup gave a residue that was chromatographed (diethyl ether/40% NH_4OH, 97:3) to give **17** as a white solid (407 mg, 93%). M.p. 116 °C, R_f = 0.42 (Et_2O/40% NH_4OH, 97:3). $[\alpha]_D^{20}$ = −36 (*c* = 1.5, $CHCl_3$). IR: ṽ = 3504, 3071, 2722, 2235, 1317 cm^{-1}. ^{1}H NMR: δ = 0.96−1.79 (m, 8 H, 4 × CH_2), 1.07 (s, 9 H, *t*Bu), 1.28 (d, *J* = 6.2 Hz, 3 H, Me), 2.37−2.55 (m, 3 H, NC*H*CH$_2$, NC*H*CH$_3$ and O*H*), 3.30−3.41 (m, 1 H, C*H*OSi), 3.47−3.60 (m, 2 H, CH_2OH), 3.75 (s, 2 H, CH_2CN), 7.35−7.47 (m, 6 H, Ar), 7.68−7.74 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 16.4 (CH_3), 19.4 (Cq), 27.0 (CH_3), 27.3, 28.2, 29.4, 33.6, 37.4 (CH_2), 58.5, 62.5 (CH), 62.6 (CH_2), 74.2 (CH), 114.7 (CN), 127.4, 127.7, 129.6, 129.7 (CHAr), 133.6, 134.6 (Cq), 135.9 (CHAr) ppm. $C_{27}H_{38}N_2O_2Si$ (450.7): calcd. C 71.95, H 8.50, N 6.22; found C 71.55, H 8.80, N 6.31.

(4*R*,6*S*,7*R*,10*R*)-7-*tert*-Butyldimethylsilyloxy-6-methyl-octahydroquinolizidine-4-carbonitrile (19): Thionyl chloride (65 μL, 0.89 mmol) was added to a solution of **17** (251 mg, 0.56 mmol) in dichloromethane (5 mL). The mixture was heated under reflux for 1.5 h and then the solvents were evaporated to dryness. Crude **18**·HCl was obtained as a brown solid that was taken up in THF (5 mL). To this solution was added LiHMDS (1 M solution in THF, 1.7 mL, 1.70 mmol) at −78 °C. The mixture was gradually warmed to room temperature and hyrolysed by the addition of an saturated aqueous solution of NH_4Cl. The usual workup followed by flash chromatography (PE/EtOAc/DCM, 90:9:1) gave quinolizidine **19** as a pale-yellow oil (216 mg, 89%). R_f = 0.71 (Et_2O/PE, 3:7). $[\alpha]_D^{20}$ = 0 (*c* = 1.2, $CHCl_3$). ^{1}H NMR: δ = 0.96 (s, 9 H, *t*Bu), 1.17 (d, *J* = 6.2 Hz, 3 H, Me), 1.20−1.75 (m, 9 H, 4.5 × CH_2), 1.85 (dq, *J* = 12.5, 2.6 Hz, 1 H, CHCNC*H*H), 2.13 (tt, *J* = 11.0, 2.4 Hz, NC*H*CC), 2.34 (qd, *J* = 8.8, 6.1 Hz, 1 H, NC*H*CH$_3$), 3.27 (ddd, *J* = 10.7, 8.8, 4.5 Hz, 1 H, C*H*OSi), 4.10 (br. t, *J*=3 Hz, 1 H, NC*H*CN), 7.27−7.33 (m, 6 H, Ar), 7.58−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 15.5 (CH_3), 19.5 (Cq), 20.2 (CH_2), 27.1 (CH_3), 28.8, 31.3, 32.7, 33.4 (CH_2), 50.1, 56.8, 63.0, 75.4 (CH), 116.7 (CN), 127.5, 127.7, 128.6, 129.7 (CHAr), 133.7, 134.7 (Cq), 135.9, 136.0 (CHAr) ppm. $C_{27}H_{36}N_2OSi$ (432.7): calcd. C 74.95, H 8.39, N 6.47; found C 74.92, H 8.45, N 6.49.

General Procedure for the Reaction of Grignard Reagents with Aminonitrile (19): A solution of the required Grignard reagent (5 equiv., 1.2 mmol) in ether was added to a 0.2-M solution of amino nitrile **19** in THF cooled to −50 °C. The mixture was stirred at room temperature until the reaction reached completion, as checked by TLC (12 h to 48 h), and then hydrolyzed by the addition of saturated aqueous NH_4Cl. The usual workup gave a residue that was purified by flash chromatography.

(3*R*,4*S*,6*R*,10*R*)-6-Ethynyl-3-*tert*-butyldimethylsilyloxy-4-methyloctahydroquinolizidine (21): Crude **20**, which resulted from the reaction of trimethylsilylethynylmagnesium bromide with **19** (0.21 mmol), was taken up in MeOH (2 mL). Potassium carbonate (40 mg, 0.29 mmol) was added and the suspension was stirred for 2 h. Water (2 mL) was added, the mixture was concentrated under reduced pressure, and then ether was added. The usual workup gave **21** as a clear oil (93 mg, quant. yield). R_f = 0.88 (Et_2O/EP, 4:6). $[\alpha]_D^{20}$ = +7 (*c* = 0.6, $CHCl_3$). ^{1}H NMR: δ = 0.97 (s, 9 H, *t*Bu), 1.18 (d, *J* = 6.2 Hz, 3 H, Me), 1.16−1.78 (m, 10 H, 5 × CH_2), 2.20 (tt, *J* = 11.1, 2.4 Hz, NC*H*CC), 2.22 (d, *J* = 2.0 Hz, 1 H, CC*H*), 2.37 (qd, *J* = 8.9, 6.2 Hz, 1 H, NC*H*CH$_3$), 3.33 (ddd, *J* = 10.7, 8.9, 4.4 Hz, 1 H, C*H*OSi), 4.10 (br. s, 1 H, NC*H*CC), 7.23−7.36 (m, 6 H, Ar), 7.59−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 14.3 (CH_3), 19.6 (Cq), 19.7 (CH_2), 27.2 (CH_3), 30.9, 31.8, 33.4, 33.9 (CH_2), 48.3, 55.1, 62.5, 73.8, 75.0 (CH), 80.9 (Cq), 127.4, 127.7, 129.5, 129.7 (CHAr), 134.0, 135.0 (Cq), 136.1 (CHAr) ppm.

(3*R*,4*S*,6*R*,10*R*)-3-*tert*-Butyldimethylsilyloxy-4-methyl-6-(phenylethynyl)octahydroquinolizidine (22): Following the general procedure above, **22** was obtained as an oil (89% yield) after flash

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chromatography (EtOAc/PE, 7:93). R_f = 0.55 (Et_2O/EP, 4:6). $[\alpha]_D^{20}$ = +4 (*c* = 3.6, $CHCl_3$). ^1H NMR: δ = 0.96 (s, 9 H, *t*Bu), 1.23 (d, *J* = 6.0 Hz, 3 H, Me), 1.06−1.85 (m, 9 H, 4.5 × C*H*$_2$), 2.29 (tt, *J* = 11.0, 2.4 Hz, NC*H*CC), 2.45 (qd, *J* = 8.9, 6.0 Hz, 1 H, NC*H*CH_3), 3.36 (ddd, *J* = 10.6, 8.9, 4.4 Hz, 1 H, C*H*OSi), 4.16 (br. t, *J*=3 Hz, 1 H, NC*H*CC), 7.22−7.40 (m, 11 H, Ar), 7.58−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 15.5 (CH_3), 19.6 (Cq), 20.0 (CH_2), 27.3 (CH_3), 31.2, 32.0, 33.6, 34.0 (CH_2), 49.0, 55.4, 62.8, 75.2 (CH), 86.5, 86.8 (Cq), 127.4, 127.7, 128.0, 128.4, 129.5, 132.0 (CHAr), 134.0, 135.2 (Cq), 136.1 (CHAr) ppm. $C_{34}H_{41}NOSi$ (507.8): calcd. C 80.42, H 8.14, N 2.76; found C 80.65, H 8.36, N 2.57.

(3*E*,3*R*,4*S*,6*R*,10*R*)-3-*tert*-Butyldimethylsilyloxy-6-(dec-3-en-1-ynyl)-4-methyloctahydroquinolizidine (23): Following the general procedure above, **23** was obtained as an oil (84% yield) after flash chromatography (EtOAc/PE, 5:95). R_f = 0.69 (Et_2O/EP, 4:6). $[\alpha]_D^{20}$ = +10 (*c* = 0.2, $CHCl_3$). ^1H NMR: δ = 0.82 (t, *J* = 6.7 Hz, 3 H, C*H*$_3$$CH_2$), 0.97 (s, 9 H, *t*Bu), 1.18 (d, *J* = 6.2 Hz, 3 H, Me), 0.80−1.73 (m, 18 H, 9 × C*H*$_2$), 2.05 (qd, *J* = 6.6, 1.5 Hz, 2 H, C*H*$_2$C=C), 2.20 (br. t, *J* = 11.0 Hz, NC*H*CC), 2.36 (qd, *J* = 9.0, 6.2 Hz, 1 H, NC*H*CH_3), 3.33 (ddd, *J* = 10.2, 9.0, 4.1 Hz, 1 H, C*H*OSi), 4.04 (br. s, 1 H, NC*H*CC), 5.48 (dd, *J* = 15.8, 1.5 Hz, 1 H, C*H*=CH), 6.08 (dt, *J* = 15.8, 6.6 Hz, 1 H, CH=C*H*), 7.22−7.34 (m, 6 H, Ar), 7.55−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 14.2, 15.5 (CH_3), 19.6 (Cq), 19.9, 22.7 (CH_2), 27.2 (CH_3), 28.9, 29.0, 31.3, 31.8, 31.9, 33.2, 33.6, 33.9 (CH_2), 48.9, 55.1, 62.6, 75.2 (CH), 85.0, 85.1 (Cq), 109.6, 127.4, 127.7, 129.5, 129.6 (CHAr), 134.0, 135.2 (Cq), 137.1 (CHAr), 144.3 (CH) ppm. $C_{36}H_{51}NOSi$ (541.9): calcd. C 79.79, H 9.49, N 2.58; found C 79.68, H 9.64, N 2.54.

(1*E*,3*R*,4*S*,6*R*,10*R*)-3-*tert*-Butyldimethylsilyloxy-6-(dec-1-enyl)-4-methyloctahydroquinolizidine (24): Sodium (5 mg, 0.22 mmol) was added to liquid ammonia (2 mL). Compound **23** (31 mg, 0.057 mmol) in THF (0.5 mL) was added to the resulting blue solution and then the mixture was cooled to −78 °C. After 5 min, the blue color faded and additional sodium (2 mg) was added. Stirring was maintained at −78 °C for 15 min and then solid NH_4Cl (100 mg) was added. The mixture was then warmed to room temperature, water and ether were added, and the usual workup gave a residue that was chromatographed (EtOAc/PE, 2:8). Compound **24** was obtained as an oil (16 mg, 51%). R_f = 0.29 (Et_2O/EP, 4:6). $[\alpha]_D^{20}$ = −11 (*c* = 0.7, $CHCl_3$). ^1H NMR: δ = 0.81 (t, *J* = 7.1 Hz, 3 H, C*H*$_3$$CH_2$), 0.96 (s, 9 H, *t*Bu), 1.13 (d, *J* = 5.8 Hz, 3 H, Me), 0.87−1.73 (m, 22 H, 11 × C*H*$_2$), 2.01 (q, *J* = 6.6 Hz, 2 H, C*H*$_2$C=C), 2.18−2.35 (m, 2 H, NC*H*CC and NC*H*CH_3), 3.32 (ddd, *J* = 10.4, 9.0, 4.4 Hz, 1 H, C*H*OSi), 3.56−3.63 (m, 1 H, NC*H*CC), 5.47 (dt, *J* = 15.3, 6.6 Hz, 1 H, C*H*=CH), 5.79 (dd, *J* = 15.3, 9.3 Hz, 1 H, CH=C*H*), 7.23−7.33 (m, 6 H, Ar), 7.57−7.64 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 14.3, 15.3 (CH_3), 19.1 (Cq), 19.6, 22.8 (CH_2), 27.2 (CH_3) 29.2, 29.5, 29.6, 32.1, 32.4, 32.8, 33.0, 44.1 (CH_2), 54.1, 56.7, 61.5, 75.4, 126.1 (CH), 127.4, 127.7, 129.5, 129.6 (CHAr), 133.4 (CH), 134.1, 135.3 (Cq), 136.1 (CHAr) ppm.

(3*R*,4*S*,6*R*,10*R*)-3-*tert*-Butyldimethylsilyloxy-6-decyl-4-methyloctahydroquinolizidine (25): Following the general procedure above, **25** was obtained as an oil (93% yield) after flash chromatography (Et_2O/PE, 2:8). R_f = 0.20 (Et_2O/EP, 4:6). $[\alpha]_D^{20}$ = −2 (*c* = 2.4, $CHCl_3$). ^1H NMR: δ = 0.90 (t, *J* = 6.7 Hz, 3 H, C*H*$_3$$CH_2$), 1.05 (s, 9 H, *t*Bu), 1.24 (d, *J* = 6.0 Hz, 3 H, Me), 1.25−1.80 (m, 28 H, 14 × C*H*$_2$), 2.24 (tt, *J* = 11.0, 2.5 Hz, NC*H*CC), 2.46 (dq, *J* = 8.6, 6.0 Hz, 1 H, NC*H*CH_3), 3.05−3.14 [br. s, 1 H, NC*H*$(CH_2)_2$], 3.41 (ddd, *J* = 10.5, 8.6, 4.0 Hz, 1 H, C*H*OSi), 4.04 (br. s, 1 H, NC*H*CC), 7.32−7.41 (m, 6 H, Ar), 7.67−7.72 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 14.3, 15.4 (CH_3), 18.2 (CH_2), 19.6 (Cq), 202, 22.8 (CH_2), 27.2 (CH_3), 27.4, 27.6, 29.5, 29.8, 30.3, 32.1, 32.8, 34.2, 34.3 (CH_2), 53.3, 53.4, 60.7, 75.6 (CH), 127.4, 127.7, 129.5, 129.6 (CHAr), 134.1, 135.3 (Cq), 136.1 (CHAr) ppm.

(3*R*,4*S*,6*R*,10*R*)-4-Methyl-3-*tert*-butyldimethylsilyloxy-6-vinyloctahydroquinolizidine (26): Silver tetrafluoroborate (13 mg, 0.067 mmol) was added to a solution of **19** (20 mg, 0.046 mmol) in THF (1 mL). The mixture was stirred at room temperature for 10 min, cooled to −78 °C and then a THF solution of vinylmagnesium bromide was added (1 M solution, 185 μL, 0.185 mmol). The mixture was warmed to room temperature and was then hydrolysed with saturated aqueous NH_4Cl. The usual workup, followed by flash chromatography, gave **26** as an oil (14 mg, 70%). R_f = 0.50 (Et_2O/EP, 4:6). ^1H NMR: δ = 0.97 (s, 9 H, *t*Bu), 1.16 (d, *J* = 6.2 Hz, 3 H, Me), 0.88−1.75 (m, 10 H, 5 × C*H*$_2$), 2.22−2.36 [m, 2 H, NC*H*CH_3 and NC*H*$(CH_2)_2$], 3.33 (ddd, *J* = 10.8, 8.9, 4.6 Hz, 1 H, C*H*OSi), 3.63−3.66 (m, 1 H, NC*H*C=CH), 5.10 (dd, *J* = 17.2, 1.5 Hz, 1 H, CH=C*H*H), 5.15 (dd, *J* = 10.6 and 1.5 Hz, 1 H, CH=CH*H*), 6.24 (dt, *J* = 17.2, 9.9 Hz, 1 H, C*H*=CHH), 7.25−7.37 (m, 6 H, Ar), 7.59−7.65 (m, 4 H, Ar) ppm. ^{13}C NMR: δ = 15.5 (CH_3), 19.5 (Cq), 19.2 (CH_2), 27.2 (CH_3), 32.4, 32.8, 34.1 (CH_2), 54.1, 57.6, 61.5, 75.4, 117.1 (CH), 127.5, 127.7, 129.5, 129.7 (CHAr), 134.4 (Cq), 135.4 (CH), 136.1 (CHAr) ppm.

General Procedure for the Deprotection of Quinolizidines 19 and 21−25: A solution of quinolizidine (0.035 mmol) and tetrabutylammonium fluoride (0.35 mmol) in THF (1 M solution, 1.5 mL) was heated at 50 °C for 24 h. Water (1 mL) was added, the aqueous layer was saturated with NaCl, and then the usual workup was applied. The crude deprotected quinolizidines were purified by flash chromatography (Et_2O then Et_2O/40% NH_4OH, 97:3). The quinolizidines were homogeneous by TLC and NMR spectroscopy, and were obtained in all cases with > 97% purity, as checked by GC.

(3*R*,4*S*,6*R*,10*R*)-6-Ethynyl-3-hydroxy-6-methyloctahydroquinolizidine (27): Yield: 68%; white solid; R_f = 0.30 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 1.23 (d, *J* = 6.2 Hz, 3 H, Me), 1.19−2.05 (m, 10 H, 5 × C*H*$_2$), 2.20−2.30 [m, 2 H, NC*H*CH_3 and NC*H*$(CH_2)_2$], 2.26 (d, *J* = 2.2 Hz, 1 H, CCH), 3.22−3.34 (m, 1 H, C*H*OH), 4.07 (br. m, 1 H, NC*H*CCH) ppm. ^{13}C NMR: δ = 14.9 (CH_3), 19.7, 30.9, 31.9, 33.6, 33.7 (CH_2), 49.3, 55.2, 62.3, 72.9, 73.9 (CH), 77.2 (Cq) ppm.

(3*R*,4*S*,6*R*,10*R*)-3-Hydroxy-4-methyl-6-phenylethynyloctahydroquinolizidine (28): Yield: 68%; white solid; R_f = 0.20 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 1.22 (d, *J* = 6.2 Hz, 3 H, Me), 1.41−1.97 (m, 10 H, 5 × C*H*$_2$), 2.29 (dq, *J* = 9.2, 6.2 Hz, 1 H, NC*H*CH_3), 2.37 [tt, *J* = 10.7, 2.2 Hz, 1H NC*H*$(CH_2)_2$], 3.25 (td, *J* = 9.7, 4.9 Hz, 1 H, C*H*OH), 4.10 (t, *J* = 2.9 Hz, 1 H, NC*H*CCH), 7.19−7.29 (m, 3 H, Ar), 7.34−7.39 (m, 2 H, Ar) ppm. ^{13}C NMR: δ = 14.9 (CH_3), 20.0, 31.1, 31.2, 33.6, 33.8 (CH_2), 48.9, 55.5, 62.6, 72.9, (CH), 86.5 (2 × Cq), 123.5 (CqAr), 128.0, 128.4, 131.9 (CHAr) ppm.

(3*E*,3*R*,4*S*,6*R*,10*R*)-6-(Dec-3-en-1-ynyl)-3-hydroxy-4-methyloctahydroquinolizidine (29): Yield: 77%; white solid; R_f = 0.20 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 0.88 (t, *J* = 6.6 Hz, 3 H, Me), 1.23 (d, *J* = 6.2 Hz, 3 H, Me), 1.10−2.03 (m, 18 H, 9 × C*H*$_2$), 2.09 (qd, *J* = 6.7, 1.5 Hz, 2 H, CH=CHC*H*$_2$), 2.20−2.40 [m, *2* H, NC*H*CH_3 and NC*H*$(CH_2)_2$], 3.22−3.34 (m, 1 H, C*H*OH), 4.14 (br. s, 1 H, NC*H*CCH), 5.50 (dq, *J*= 15.8, 1.5 Hz, 1 H, CCC*H*=CH), 6.11 (dt, *J* = 15.8, 6.7 Hz, 1 H, CCCH=C*H*) ppm. ^{13}C NMR: δ = 14.3, 15.0 (CH_3), 20.0, 22.8, 29.0, 29.9, 31.2, 31.8, 32.0, 33.2, 33.8 (CH_2), 48.9, 55.3, 62.4, 73.1, (CH), 84.8, 85.1 (Cq), 109.4, 144.5 (CH) ppm.

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(1*E*,3*R*,4*S*,6*R*,10*R*)-6-(Dec-1-enyl)-3-hydroxy-4-methyloctahydroquinolizidine (30): Yield: 65%; white solid; R_f = 0.20 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 0.88 (t, *J* = 6.6 Hz, 3 H, Me), 1.20 (d, *J* = 6.0 Hz, 3 H, Me), 1.23−2.17 (m, 25 H, 12 × C*H*$_2$ and NC*H*CH_3), 2.44 [tt, *J* = 10.5, 2.6 Hz, 1 H, NC*H*$(CH_2)_2$], 3.29 (td, *J* = 9.5, 3.3 Hz, 1 H, C*H*OH), 3.70 (dt, *J* = 9.2, 3.3 Hz, 1 H, NC*H*CCH), 5.56 (dt, *J*= 15.5, 6.6 Hz, 1 H, CHCH=C*H*), 5.85 (ddt, *J* = 15.5, 9.2, 1.1 Hz, 1 H, CHC*H*=CH) ppm. ^{13}C NMR: δ = 14.3, 14.8 (CH_3), 19.1, 22.8, 29.3, 29.6, 29.9, 32.1, 32.3, 32.8, 33.8, 34.1 (CH_2), 54.3, 56.7, 61.4, 73.2, (CH), 125.6, 133.9 (CH) ppm.

(3*R*,4*S*,6*R*,10*R*)-6-Decyl-3-hydroxy-4-methyloctahydroquinolizidine (31): Yield: 76%; white solid; R_f = 0.20 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 0.88 (t, *J* = 6.6 Hz, 3 H, Me), 1.20 (d, *J* = 6.0 Hz, 3 H, Me), 1.05−2.00 (m, 28 H, 14 × C*H*$_2$), 2.22−2.37 [m, 2H NC*H*$(CH_2)_2$ and NC*H*CH_3], 3.09−3.16 [m, 1 H, NC*H*$(CH_2)_2$], 3.29 (td, *J* = 9.7, 4.7 Hz, 1 H, C*H*OH) ppm. ^{13}C NMR: δ = 14.3, 14.8 (CH_3), 18.2, 20.3, 22.8, 27.3, 27.6, 29.5, 29.8, 29.9, 30.2, 32.1, 32.7, 33.9, 34.3 (CH_2), 53.4 (2 × CH), 60.5, 73.2 (CH) ppm.

(4*R*,6*S*,7*R*,10*R*)-7-Hydroxy-6-methyloctahydroquinolizidine-4-carbonitrile (32): Yield: 84%; white solid; R_f = 0.20 (Et_2O/40% NH_4OH, 97:3). ^1H NMR: δ = 1.24 (d, *J* = 6.2 Hz, 3 H, Me), 1.10−1.20 (m, 10 H, 5 × C*H*$_2$), 2.17 (qd, *J* = 9.2, 6.1 Hz, 1 H, NC*H*CH_3), 2.26 (tt, *J* = 10.5, 2.2 Hz, NC*H*CC), 3.22 (td, *J* = 9.2, 4.5 Hz, 1 H, C*H*OH), 4.21 (br. t, *J* = 3.4 Hz, 1 H, NC*H*CN) ppm. ^{13}C NMR: δ = 15.1 (CH_3), 20.3, 28.8, 31.5, 32.8, 33.5 (CH_2), 50.1, 56.6, 63.0, 75.2 (CH), 116.7 (CN) ppm.

Pharmacology

Four human-tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These lines included one glioblastoma (U-373 MG), one colon (HCT-15), one non-small-cell lung (A549), and one bladder (J82) cancer model. The ATCC numbers of these cell lines are HTB 17 (U-373 MG), CCL225 (HCT-15), CCL 185 (A549) and HTB1 (J82). The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco) supplemented with 5% fetal-calf serum (FCS). All the media were supplemented with a mixture of glutamine (Gibco, 0.6 mg/mL), penicillin (Gibco, 200 IU/mL), streptomycin (Gibco, 200 IU/mL) and gentamycin (Gibco, 0.1 mg/mL). The FCS was heat-inactivated for 1 h at 56 °C.

The four cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 40,000 cells/mL culture medium) to ensure adequate plating prior to the determination of the cell growth. This process was carried out by means of the colourimetric MTT assay, as detailed previously.[16,17] This assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT [3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St Louis, MO] to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells is directly proportional to the intensity of the blue colour, which is quantitatively measured by spectrophotometry on a DIAS microplate reader (Dynatech Laboratories, Guyancourt, France) at a 570 nm wavelength (with a reference of 630 nm). Each experiment was carried out in sextuplicate. We validated the MTT-related data by using two alternative techniques, namely direct cell counting and the genomic incorporation of tritiated thymidine (data not shown).

Five concentrations ranging from 10^{-5} to 10^{-7} M were assayed for six of the nine compounds (three reference and six investigational) under study (see Table 1).

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