HR22C16: A Potent Small Molecule Probe for the Dynamics of Cell Division

Srinivas Hotha, Justin C. Yarrow, Janet G. Yang, Sarah Garrett, Kishore V. Renduchintala, Thomas U. Mayer and Tarun M. Kapoor*

Laboratory of Chemistry and Cell Biology, The Rockefeller University, 1230 York Ave, New York, NY 10021
Department of Cell Biology, Harvard Medical School, Boston, MA 02115
Max Planck Institute for Biochemistry, Am Klopferspitz 18a, 82152 Martinsried/Munich, Germany

Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Experimental Techniques and Apparatus</td>
<td>2</td>
</tr>
<tr>
<td>Experimental Procedures: Solution Phase</td>
<td>3</td>
</tr>
<tr>
<td>Experimental Procedures: Solid Phase</td>
<td>6</td>
</tr>
<tr>
<td>Biological Evaluation of Compounds</td>
<td>8</td>
</tr>
<tr>
<td>Characterization Data</td>
<td>10</td>
</tr>
<tr>
<td>Mass Spectral Data of Library Members</td>
<td>11</td>
</tr>
<tr>
<td>LC Analysis of HR22C16 Analogs</td>
<td>12</td>
</tr>
<tr>
<td>NMR Spectra</td>
<td>16</td>
</tr>
<tr>
<td>References</td>
<td>19</td>
</tr>
</tbody>
</table>
General Experimental Techniques and Apparatus

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Anhydrous grade CH$_2$Cl$_2$ and $N, N'$-dimethylformamide (DMF) were purchased from Aldrich and stored over 4 Å molecular sieves. Anhydrous grade methyl sulfoxide (DMSO) and tetrahydrofuran (THF) were purchased from Aldrich and directly used. Unless otherwise reported all reactions were performed under nitrogen atmosphere. Solid phase reactions were carried out in screw capped vials or glass culture tubes. Removal of solvent in vacuo refers to distillation using a Buchi rotary evaporator attached to an efficient vacuum pump. Products obtained as solids or syrups were dried under high vacuum. Analytical thin-layer chromatography was performed on pre-coated silica plates (Whatman F254, 0.25 mm thickness); compounds were visualized by UV light or by staining with anisaldehyde spray or ninhydrin. $^1$H, 2D-NOESY-NMR spectra were recorded on Bruker DPX 400 (400 MHz for $^1$H and 100 MHz for $^{13}$C NMR) spectrometer. Chemical shifts ($\delta_H$) are quoted in ppm and are referenced to tetramethylsilane (internal). LC-MS data was obtained using Waters LC-MS ZQ 2795 spectrometer equipped with Waters symmetry C18 2.1 x 50 mm column at a flow rate of 0.2 mL/min. Photolysis was carried out using KIMMON IK-Series Helium-Cadmium laser system (325 nm wavelength). BS-C-1 (monkey kidney epithelial) cells and human tumor cell line were cultured in DMEM high glucose medium, supplemented with 10% fetal calf serum (FCS) and 100 U/mL penicillin and streptomycin. The cells were maintained at 37 °C and 5 % CO$_2$. Immunofluorescence staining of cells was observed using Zeiss Axiovert 200M or Axioplan2 microscope.
Experimental Procedures: Solution Phase

Synthesis of $\text{N}_b$-[tert-butoxycarbonyl]-$\text{N}_b$-allyl-L-tryptophan (2d)

To a solution of L-tryptophan methylester (3.0 g, 13.8 mmol) in 5 mL of THF was added a solution of allyl bromide (1.2 mL, 13.8 mmol) in THF (5 mL) at 0 °C and stirred under nitrogen atmosphere at room temperature for 24 h by which time a thick precipitate has formed. The solid was dissolved by adding 2 mL of aq. ammonium hydroxide and the resulting solution was diluted with water and extracted three times with 25 mL portions of ethyl acetate. Combined organic layers were washed with brine solution, dried (anhy. Na$_2$SO$_4$), concentrated under reduced pressure and the residue was directly used for tert-butoxycarbonyl protection.

To a solution of $\text{N}_b$-allyl L-tryptophan methyl ester (2.9 g, 11.2 mmol) in 15 mL of THF was added sequentially di t-butyldicarbonate (3.7 g, 16.9 mmol), triethyl amine (4.9 mL, 33.6 mmol) and catalytic amount of $N,N'$-dimethylaminopyridine and stirred at room temperature. At the end of 48 h, the reaction mixture was concentrated and the residue was redissolved in 30 mL of ethyl acetate and washed with water. The organic layer was washed with brine solution, dried (anhy. Na$_2$SO$_4$), concentrated under reduced pressure and the residue was directly taken for the next step without further purification.

$\text{N}_b$-[tert-butoxycarbonyl]-$\text{N}_b$-allyl L-tryptophan methyl ester (3.2 g, 8.9 mmol) was dissolved in 10 mL of THF and was added 1 N aq. LiOH solution (5 mL) and stirred at room temperature for 36 h. The resulting reddish brown residue was diluted with ethyl acetate, washed with 1 M aq. oxalic acid solution, the organic layer was washed with brine solution, dried over anhydrous Na$_2$SO$_4$ and concentrated to dryness in vacuo. The brown colored residue was purified by conventional silica gel column chromatography using 1:10 methanol-chloroform mixture to afford the $\text{N}_b$-[tert-butoxycarbonyl]-$\text{N}_b$-allyl L-tryptophan (2d) (2.95 g, 62 % overall) as pale yellow colored solid. The same set of reactions was repeated to get $\text{N}_b$-[tert-butoxycarbonyl]-$\text{N}_b$-(Z-2'-iodo-2'-butenyl) L-tryptophan (2e) using Z-2'-iodo-2'-butenyl bromide.[1]

Synthesis of trans-5-[meta-hydroxyphenyl]-2-n-propyl-1, 3-dioxo-6H-1, 2, 3, 5, 11, 11a-hexa-hydroimidazo[1,5-b]-β-carboline (8) and cis-5-[meta-hydroxyphenyl]-2-n-propyl-1, 3-dioxo-6H-1, 2, 3, 5, 11, 11a-hexahydro-imidazo[1,5-b]-β-carboline (9)

The reaction mixture consisting of L-tryptophan (2.0 g, 9.8 mmol), 3-hydroxybenzaldehyde (1.32 g, 10.8 mmol), 10 mL of 1 N H$_2$SO$_4$, 30 mL of H$_2$O and 4 mL of ethanol (95 %) was stirred at 60 °C for four hours.[2a] After the addition of 20 mL of ammonium hydroxide (aq), the pale yellow colored solution was cooled in an ice-bath and extracted with two 50 mL portions of ether. The aqueous solution was then concentrated to one third the volume and then cooled at 4 °C for 15 h. The resulting solid was filtered by suction, washed with cold water, dried at 80 °C for 12 h. Powdered tetrahydro β-carboline product (100 mg, 0.3 mmol) was suspended in 5 mL of THF and propyl isocyanate (33 µL, 0.33 mmol) was added.[2b] After refluxing for 24 h, the
remaining starting material was removed by filtration and the filtrate was concentrated in vacuo. Compounds 8 and 9 were purified by conventional silica gel column chromatography using 1:3 ethyl acetate/petroleum ether and concentrated in vacuo to get a pale yellow colored thick syrup whose 1H NMR and 2D-NOESY spectral data (Charts attached) were in conformity with those of assigned structures. Overall yields for compounds 8 and 9 are 34% and 37%, respectively. 71% is the overall yield for both isomers.

*Trans*-5-[meta-hydroxyphenyl]-2-*n*-propyl-1, 3-dioxo-6H-1, 2, 3, 5, 11, 11a-hexahydroimidazo[1,5-b]-β-carboline (8)

1H NMR (400 MHz, CDCl3): δ = 0.86 (t, J = 7.4 Hz, 3 H), 1.58 (q, J = 7.3 Hz, 2 H), 2.79 (dd, J = 1.6, 11.2 Hz, 1 H), 3.39 (m, 3 H), 4.22 (dd, J = 5.8, 11.6 Hz, 1 H), 6.14 (s, 1 H), 6.73 (m, 2 H), 6.83 (m, 2 H), 7.17 (m, 4 H), 7.50 (d, J = 7.76 Hz, 1 H), 8.33 (s, 1 H).

ESMS calcd for C22H21N3O3: m/z 375.16, found 374.19 (M-1).

*Cis*-5-[meta-hydroxyphenyl]-2-*n*-propyl-1, 3-dioxo-6H-1, 2, 3, 5, 11, 11a-hexahydroimidazo[1,5-b]-β-carboline (9)

1H NMR (400 MHz, CDCl3): δ = 0.92 (m, 3 H), 1.64 (m, 2 H), 3.07 (m, 2 H), 3.46 (m, 4 H), 4.42 (m, 1 H), 5.77 (s, 1 H), 6.75 (m, 3 H), 7.12 (m, 3 H), 7.85-7.40 (m, 2 H). ESMS calcd for C22H21N3O3: m/z 375.16, found 374.18 (M-1).

**Synthesis of caged HR22C16 analog 7**

To a stirred solution of 3-hydroxybenzaldehyde (261 mg, 2.14 mmol) in 4 mL of anhydrous DMF was added sequentially 2-nitrobenzyl bromide (462 mg, 2.14 mmol), potassium carbonate (591 mg, 4.28 mmol) and tetrabutylammonium iodide (790 mg, 2.14 mmol). The resulting reaction mixture was stirred at 100 °C for 1 h, allowed to cool to room temperature, poured into water, extracted with ethyl acetate (3 x 25 mL). Combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to obtain 3-[(2-nitrophenyl)methoxy]-benzaldehyde (530 mg, 96 %) as a white solid that was directly used in the next step.

To a solution of L-tryptophan methylester (93 mg, 0.42 mmol) in tetrahydrofuran (THF) containing 5 % trifluoroacetic acid (TFA) was added above prepared aldehyde (100 mg, 0.39 mmol) and heated to reflux. At the end of 15 h, the reaction mixture was neutralized using aq. NH4OH solution, diluted with water and extracted with ethyl acetate (3 x 25 mL). Combined organic layers were given brine solution wash, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to obtain crude product that was purified by conventional silica gel column chromatography to obtain tetrahydro β-carboline derivative (170 mg, 89 %) that was taken directly for the next step. To a THF (5 mL) solution of tetrahydro β-carboline derivative (36 mg, 79 µmol) synthesized vide supra was added n-butyl isocyanate (18 µL, 158 µmol) and refluxed for 15 h.[2b] The reaction mixture was concentrated to dryness and purified by silica gel column chromatography to obtain caged HR22C16 analog 7 (16 mg, 39 %).

1H NMR (400 MHz, CDCl3): δ = 0.91 (t, J = 7.5 Hz, 3 H), 1.33 (m, 2 H), 1.62 (m, 2 H), 2.91 (m, 1 H), 3.54 (m, 3 H), 4.33 (m, 1 H), 5.43 (s, 2 H), 6.27 (s, 1 H), 6.95 (m, 3 H),
7.10-7.32 (m, 5 H), 7.45-7.65 (m, 3 H), 7.81-8.10 (m, 2 H); ESMS calcd. for C_{30}H_{28}N_{4}O_{5}: m/z 524.57, found 525.51 (M+1).

**Uncaging of HR22C16 analog (7) by photolysis reaction**

A THF (1.14 mL) solution of compound 7 (6 mg, 11.5 µmol) in a plastic eppendorf tube was irradiated for 45 sec using He-Cd laser at room temperature. Resulting pale yellow colored solution was concentrated *in vacuo* and purified by silica gel column chromatography to obtain uncaged HR22C16 (1).

^{1}H NMR (400 MHz, CDCl_{3}): \( \delta = 0.92 \) (t, \( J = 7.3 \) Hz, 3 H), 1.32 (m, 2 H), 1.59 (m, 2 H), 2.88 (dd, \( J = 9.7, 14.5 \) Hz, 1 H), 3.51 (m, 3 H), 4.30 (dd, \( J = 5.5, 10.9 \) Hz, 1 H), 6.24 (s, 1 H), 6.81 (s, 2 H), 6.87 (d, \( J = 7.4 \) Hz, 1 H), 7.15-7.31 (m, 5 H), 7.55 (d, \( J = 7.5 \) Hz, 1 H), 7.84 (s, 1 H). ESMS calcd for C_{23}H_{23}N_{3}O_{3}: m/z 389.45, found 390.57 (M+1).
Experimental Procedures: Solid Phase

Loading of \( N_b\text{-[tert-butoxycarbonyl]-N}_b\text{-allyl L-tryptophan onto solid support} \)

To a solution of \( N_b\text{-[tert-butoxycarbonyl]-N}_b\text{-allyl L-tryptophan (2d)} \) (366 mg, 1.06 mmol) in CH\(_2\)Cl\(_2\) was added 1, 1’-Carbonyldiimidazole (171 mg, 1.05 mmol) and stirred for 1 h at room temperature under nitrogen atmosphere. The resulting solution was added to a pre-swelled (CH\(_2\)Cl\(_2\)) Novasyn TG-S-OH resin (0.95 g, (Calbiochem-Novabiochem, 0.26 mmol/g)) and shaken on a rotator for 15 h at room temperature. The beads were collected by filtration, washed with CH\(_2\)Cl\(_2\) (4 x 5 mL), DMF (4 x 5 mL), 1:1 DMF-MeOH (2 x 5 mL), MeOH (2 x 5 mL) and dried in vacuo for 2 h. A small portion of these beads was cleaved to determine loading efficiency.

Checking the progress of the reaction on solid-phase

Progress of a solid-phase reaction was checked by cleaving the substrate from the beads by incubating the resin in a solution of 1:4 0.5 % aq. NaOH/2-propanol at 50 ºC for 2 h. Resin was removed by filtration, the filtrate was concentrated in vacuo and analyzed by LC-MS. This procedure was carried out at various stages of the synthesis protocol.

Solid-phase diastereoselective Pictet-Spengler reaction

To a suspension of \( N_b\text{-[tert-butoxycarbonyl]-N}_b\text{-allyl L-tryptophan-resin (3d)} \) (ca. 400 mg, 104 µmol) in 6 mL of 5 % TFA/CH\(_2\)Cl\(_2\) was added aldehyde (20 eq). After the mixture was allowed to react at 50 ºC for 24 h, the resin was filtered off, washed with CH\(_2\)Cl\(_2\) (3 x 5 mL), DMF (3 x 5 mL), 1:1 DMF-MeOH (3 x 5 mL), MeOH (2 x 5 mL) and dried in vacuo for 2 h. A portion of the resin was saved to check the progress of the reaction. For reactions where Pictet-Spengler reaction was observed (by cleaving the substrate from the solid support) to be incomplete, the resin was treated with propyl isocyanate (10 eq) at 50 ºC for 24 h and then, the resin was filtered and worked up as above.

It has been envisaged that the alkaline conditions employed for cleavage of the substrate from tenta gel resin will not be compatible (possibility of undesired ester hydrolysis) when the 5-oxo-2, 2-bis(phenylthio)-pentanoic acid methyl ester (R\(^1\))\(^3\) was used as the aldehyde. Thus, hydroxymethyl Wang resin was used instead of Novasyn TG-S-OH resin. Loading of the \( N_b\text{-[tert-butoxycarbonyl]-N}_b\text{-(Z-2'-iodo-2'-butenyl) L-tryptophan (2e)} \) and the Pictet-Spengler reaction were carried out by using protocols mentioned above. At the end of the reaction, the resin was filtered off and washed with CH\(_2\)Cl\(_2\) (3 x 5 mL), DMF (3 x 5 mL), 1:1 DMF-MeOH (3 x 5 mL), MeOH (2 x 5 mL) and dried in vacuo for 2 h. Progress of the reaction was checked by cleaving the substrate from beads by incubating the resin with trifluoroacetic acid at room temperature for 1 h. Resin was removed by filtration, the filtrate was concentrated in vacuo and analyzed by LC-MS.

Deallylation\(^4\)

Resin 4d (200 mg, 52 µmol) was treated at 50 ºC for 6 h in a solution of \( N, N'\text{-dimethylbarbituric acid (46.8 mg, 300 µmol) and tetrakis(triphenylphosphine) palladium (23.2 mg, 20 µmol) in 4 mL of dried degassed CH\(_2\)Cl\(_2\) under nitrogen. The resin was collected by filtration and washed with CH\(_2\)Cl\(_2\) (3 x 5 mL), 1:9 trifluoroacetic
acid/CH$_2$Cl$_2$ (2 x 5 mL), CH$_2$Cl$_2$ (3 x 5 mL), 1:9 triethylamine/CH$_2$Cl$_2$ (2 x 5 mL), CH$_2$Cl$_2$ (3 x 5 mL), DMF (3 x 5 mL), 1:1 DMF/MeOH (3 x 5 mL), MeOH (2 x 5 mL). Finally, the resin was dried under high vacuum for 2 h and was directly used in the next step. A small portion of beads was cleaved to identify the purity of the substrate on the solid support by using the standard procedure detailed above.

**Hydantoination and “traceless” cleavage from solid support into formats compatible with cell-based assays**

Resin obtained from the previous step was arrayed into 96-well plate and incubated at 55 °C in THF with appropriate isocyanate$^5$ (10 eq). After 36 h, solutions with cleaved compounds were transferred into another 96-well plate. The remaining resin was washed with THF (2 mL), CH$_2$Cl$_2$ (2 mL) and the solution was transferred to the corresponding wells in the 96-well plate. The arrayed solutions were incubated with scavenger amine resin (40 mg, 45 µmol) to remove excess unreacted isocyanates. After 24 h, again the solution was filtered into another 96-well daughter plate and evaporated to dryness. Compounds wherein BocNH(CH$_2$)$_5$NCO was used were transferred into another well for Boc- deprotection using equal volume of 20 % TFA in CH$_2$Cl$_2$ for 4 h, followed by evaporation. 100 µL of anhydrous DMSO was added into each well, was analyzed by spectrophotometry to get the exact concentration followed by required dilution to make a stock solution of 10 mM concentration. A small library of 50 compounds was synthesized using various aldehydes and isocyanates by adopting this protocol. LC-MS analysis was carried out for all compounds (representative LC traces are shown in Figure 3). The major peaks correspond to the trans- products, confirmed by $^1$H NMR in several cases (Figure 6, 7 provide examples).
Biological Evaluation of Compounds

Primary cell-based chemical genetic screen

BS-C-1 epithelial cells were grown in 384 well clear bottomed plates. Cell monolayers were wounded and compounds from an unbiased library of 16,000 members (Diverset E, Chembridge Corporation) were transferred to give an estimated concentration of 30 µM. Actin was stained with Rhodamine-Phalloidin (Sigma) under standard conditions. Plates were incubated for 7 h prior to fixation and processing for imaging.

Cell –Based assays for HR22C16 analogs

To assess the effect of HR22C16 analogs, vertebrate cells (BS-C-1) plated on coverslips were treated for 4 h in normal growth medium containing a final concentration of 25 µM HR22C16 analogs and the percentage of mitotics was tabulated (Table 1). In vivo anti-mitotic activity of caged (7) and uncaged (1) compounds was determined by incubating a human tumor cell line for 4 hours in normal growth medium containing 5 µM DMSO solution of respective compounds. Coverslips were processed for immunofluorescence[6] and cells in interphase or mitosis were counted. Two independent experiments were carried out and the average value was taken.

Table 1. Mitotic index of vertebrate cells treated with HR22C16 analogs

<table>
<thead>
<tr>
<th>R1</th>
<th>OH</th>
<th></th>
<th></th>
<th>NO2</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.3</td>
<td>10.6</td>
<td>2.6</td>
<td>4.3</td>
<td>2.4</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>5.0</td>
<td>2.8</td>
<td>4.3</td>
<td>2.5</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>9.4</td>
<td>3.3</td>
<td>3.2</td>
<td>2.3</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.9</td>
<td>11.2</td>
<td>4.4</td>
<td>5.0</td>
<td>2.7</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.9</td>
<td>12.6</td>
<td>4.3</td>
<td>4.7</td>
<td>2.5</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In vitro motility Assays for the mitotic kinesin, Eg5

Motility assays were performed according to the procedure described by Kapoor and Mitchison.[7] Compounds 1 and 6 were serially diluted in DMSO to desired concentrations, and added to assays at a final DMSO concentration of 5 %. Motility assays of caged (7) and uncaged (1) compounds were carried out by serially diluting 10 mM solutions of respective compounds in DMSO to 2.5 µM concentration and added to assays.[7] Velocities were measured using MetaMorph software (Universal Imaging Corporation). All velocity data represent the results from two or more experiments, and the average percent inhibition or the relative velocity is shown with the SE. Graphs were prepared using Kaleidagraph software and IC50 for compound 1: 800±10 nM; compound 6: 90 ± 40 nM were determined using the software and as described in reference 6 (Figure 1). For compound 6, the following concentrations were tested: 0 µM, 0.02 µM, 0.10 µM, 0.17 µM, 0.25 µM, 0.50 µM, 2.50 µM, 12.50 µM.
Figure 1

% inhibition vs. [compound 1] (M)

% inhibition vs. [compound 6] (M)
Compounds from each well after the completion of the synthesis were analyzed by LC-MS (Table 2). In order to examine the diastereoselectivity, compound A1 was analyzed by LC-MS. For standards, trans- and cis- isomers (8 and 9) were synthesized in solution by a non-diastereoselective Pictet-Spengler reaction (LC-MS traces attached, 2D-NMR analysis was used to confirm the assignment of these compounds (Figure 8, 9)). The LC method was optimized such that trans- (8) and cis- (9) isomers of A1 were well resolved. Using the software, peak areas were determined by integration of the LC trace, the observed diastereoselectivity for the solid-phase reaction was found to be more than 9:1 (trans:cis) (Figure 2). Diastereoselectivity for all other library members was determined using the LC method optimized for A1 (representative traces for several compounds are shown in Figure 3). Figure 4 shows the LC data for the cleavage product from the Pictet-Spengler reaction on tenta gel bound \(N_b-(\text{tert-butoxycarbonyl})-N_b-(Z-2'-iolo-2'-butenyl)\) L-tryptophan (3e) and the 3-hydroxybenzaldehyde. Figure 5 shows the LC trace of the cleavage product from the Pictet-Spengler reaction on Wang resin bound \(N_b-(\text{tert-butoxycarbonyl})-N_b-(Z-2'-iolo-2'-butenyl)\) L-tryptophan and 5-oxo-2, 2-bis(phenylthio)-pentanoic acid methyl ester (R'). In several cases \(^1\)H NMR was used to ensure that only one diastereomer was synthesized by the solid-phase method; \(^1\)H NMR spectra of C2 and D5 are presented as representative examples (Figure 6, 7).
# Mass Spectral Data of Library Members

## Table 2. Mass spectral data and compound identification

![Structure](image)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>19</td>
<td>16</td>
<td>25</td>
<td>(A1)</td>
<td>15</td>
<td>24</td>
<td>(A2)</td>
<td>13</td>
<td>25</td>
<td>(A3)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>24</td>
<td>(B1)</td>
<td>15</td>
<td>26</td>
<td>(B2)</td>
<td>13</td>
<td>25</td>
<td>(B3)</td>
<td>19</td>
<td>30</td>
<td>(B4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>18</td>
<td>14</td>
<td>25</td>
<td>(C1)</td>
<td>12</td>
<td>25</td>
<td>(C2)</td>
<td>19</td>
<td>29</td>
<td>(C3)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>34</td>
<td>26</td>
<td>34</td>
<td>(D1)</td>
<td>24</td>
<td>34</td>
<td>(D2)</td>
<td>30</td>
<td>44</td>
<td>(D3)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>22</td>
<td>17</td>
<td>29</td>
<td>(E1)</td>
<td>15</td>
<td>23</td>
<td>(E2)</td>
<td>23</td>
<td>34</td>
<td>(E3)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>33</td>
<td>21</td>
<td>45</td>
<td>(F1)</td>
<td>19</td>
<td>28</td>
<td>(F2)</td>
<td>25</td>
<td>34</td>
<td>(F3)</td>
<td>25</td>
</tr>
</tbody>
</table>

*Numbers in parenthesis indicate the compound identity in LC traces (attached)*
LC Analysis of HR22C16 Analogs

Figure 2. LC trace of the final product (A1) from solid-phase synthesis (trace 1 from top), equimolar mixture of compounds 8 & 9 (trace 2), Compound 9 (trace 3) and compound 8 (trace 4)
Figure 3. Representative LC traces for compounds synthesized on solid-phase
Figure 4. LC trace* of cleavage product from the Pictet-Spengler reaction on tentagel resin bound N_b-(tert-butoxycarbonyl)-N_b-(Z-2'-iodo-2'-butenyl) L-tryptophan (3e) and 3-hydroxybenzaldehyde and the lower trace shows the cleavage product after deallylation

* Peak at a R_t of 7.67 corresponds to the starting material and that of 11.54 is the desired tetrahydrobetacarboline (upper trace)

Figure 5. LC trace* of cleavage product from the Pictet-Spengler reaction on Wang resin bound N_b-(tert-butoxycarbonyl)-N_b-(Z-2'-iodo-2'-butenyl) L-tryptophan and 5-oxo-2, 2-bis(phenylthio)-pentanoic acid methyl ester

* Peak at a R_t of 13.64 corresponds to the thiophenol eliminated compound and that of 14.01 is the desired Pictet-Spengler reaction product
NMR Spectra

Figure 6. $^1$H-NMR spectrum of library member C2

Figure 7. $^1$H-NMR spectrum of library member D5
Figure 8. 2D-NOESY spectrum of trans-propyl HR22C16 analog (8)

NOE crosspeak between H-1 (δ_H 6.14 ppm) and H-3 (δ_H 4.22 ppm) was absent
Figure 9. 2D-NOESY spectrum of cis-propyl HR22C16 analog (9)

Observe NOE cross peak between H-1 ($\delta_H$ 5.77 ppm) and H-3 ($\delta_H$ 4.42 ppm)
References


