Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z51173

69451 Weinheim, Germany

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

General Experimental Techniques and Apparatus

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Anhydrous grade CH_2Cl_2 and *N*, *N'*-dimethylformamide (DMF) were purchased from Aldrich and stored over 4 Aº 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 (δ_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 ^{o}C and 5 % CO_2. Immunofluorescence staining of cells was observed using Zeiss Axiovert 200M or Axioplan2 microscope.

Experimental Procedures: Solution Phase

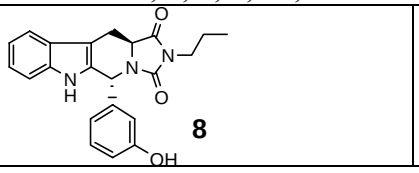
Synthesis of N_b-[*tert*-butoxycarbonyl]-N_b-allyl-L-tryptophan (2d)

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 ^{o}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_2SO_4), concentrated under reduced pressure and the residue was directly used for *tert*-butoxycarbonyl protection.

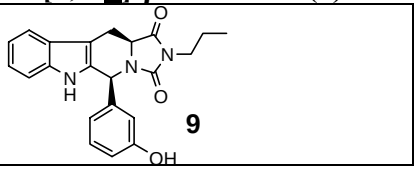
To a solution of N_b-allyl L-tryptophan methyl ester (2.9 g, 11.2 mmol) in 15 mL of THF was added sequentially di *t*-butyldicarbonate (3.7 g, 16.9 mmol), triethyl amine (4.9 mL, 33.6 mmol) and catalytic amount of *N*, *N'*-dimethylaminopyridine and stirred at room temperature. At the end of 48 h, the 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_2SO_4), concentrated under reduced pressure and the residue was directly taken for the next step without further purification.

N_b-[*tert*-butoxycarbonyl]-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_2SO_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 N_b-[*tert*-butoxycarbonyl]-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 N_b-[*tert*-butoxycarbonyl]-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)



8



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_2SO_4, 30 mL of H_2O and 4 mL of ethanol (95 %) was stirred at 60 ^{o}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 ^{o}C for 15 h. The resulting solid was filtered by suction, washed with cold water, dried at 80 ^{o}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 ^{1}H 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**)
^{1}H NMR (400 MHz, $CDCl_3$): 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 $C_{22}H_{21}N_3O_3$: *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**)
^{1}H NMR (400 MHz, $CDCl_3$): 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 $C_{22}H_{21}N_3O_3$: *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 ^{o}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. NH_4OH 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 %).

^{1}H NMR (400 MHz, $CDCl_3$): 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 $C_{30}H_{28}N_4O_5$: *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, $CDCl_3$): δ = 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 $C_{23}H_{23}N_3O_3$: *m/z* 389.45, found 390.57 (M+1).

Experimental Procedures: Solid Phase

Loading of N_b-[*tert*-butoxycarbonyl]-N_b-allyl L-tryptophan onto solid support

To a solution of N_b-[*tert*-butoxycarbonyl]-N_b-allyl L-tryptophan (**2d**) (366 mg, 1.06 mmol) in CH_2Cl_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_2Cl_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_2Cl_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-[*tert*-butoxycarbonyl]-N_b-allyl L-tryptophan-resin (**3d**) (ca. 400 mg, 104 μmol) in 6 mL of 5 % TFA/CH_2Cl_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_2Cl_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-[*tert*-butoxycarbonyl]-N_b-(*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_2Cl_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*'-dimethylbarbituric acid (46.8 mg, 300 μmol) and tetrakis(triphenylphosphine) palladium (23.2 mg, 20 μmol) in 4 mL of dried degassed CH_2Cl_2 under nitrogen. The resin was collected by filtration and washed with CH_2Cl_2 (3 x 5 mL), 1:9 trifluoroacetic

acid/CH_2Cl_2 (2 x 5 mL), CH_2Cl_2 (3 x 5 mL), 1:9 triethylamine/CH_2Cl_2 (2 x 5 mL), CH_2Cl_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 ^oC 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_2Cl_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 $BocNH(CH_2)_5NCO$ was used were transferred into another well for Boc- deprotection using equal volume of 20 % TFA in CH_2Cl_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 ^1H NMR in several cases (Figure 6, 7 provide examples).

Biological Evaluation of Compounds

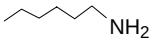
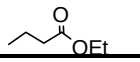
Primary cell-based chemical genetic screen

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.

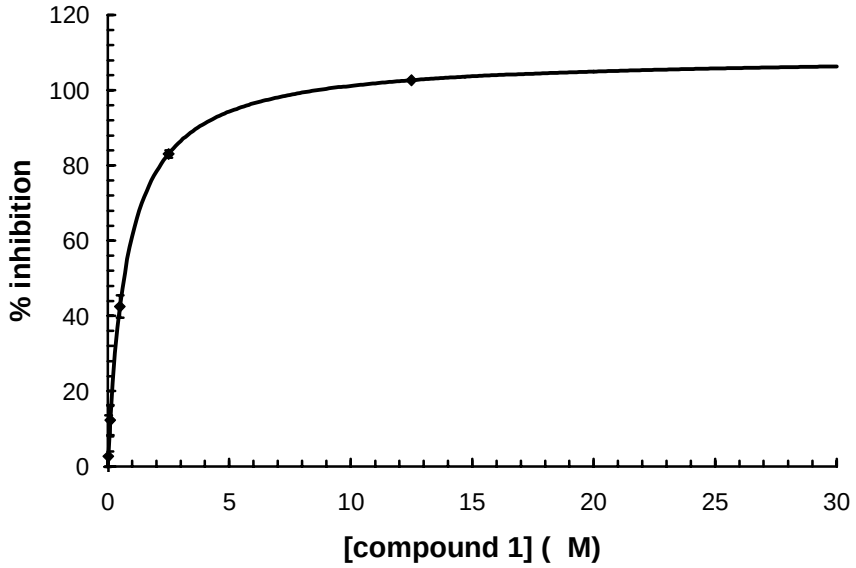
Table 1. Mitotic index of vertebrate cells treated with HR22C16 analogs

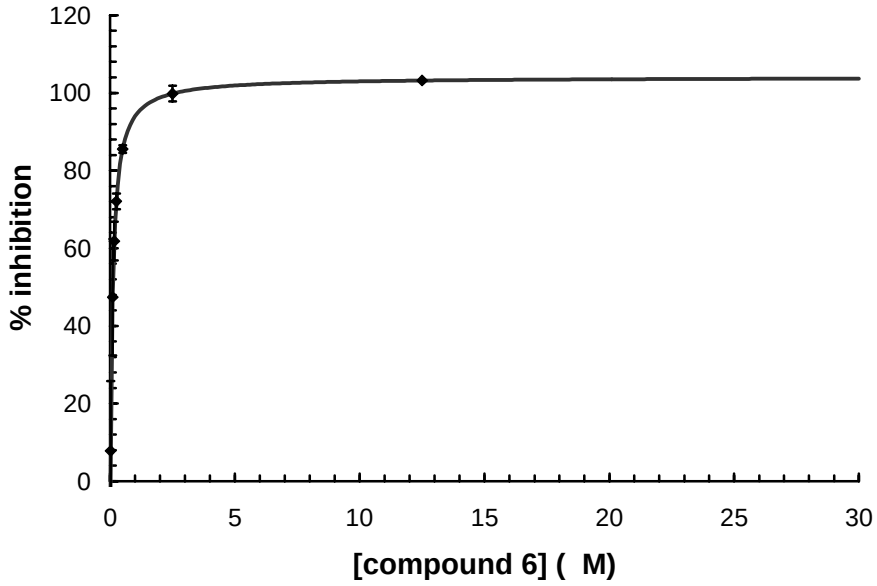
R_2 \ R_1						
	**12.3**	**10.6**	**2.6**	**4.3**	**2.4**	7.4
	**8.4**	**5.0**	**2.8**	**4.3**	**2.5**	4.3
	**11.2**	**9.4**	**3.3**	**3.2**	**2.3**	10.4
	**13.9**	**11.2**	**4.4**	**5.0**	**2.7**	11.3
	15.9	12.6	4.3	4.7	2.5	4.2

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 IC_{50} 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


120
100
80
60
40
20
0
% inhibition
0
5
10
15
20
25
30
[compound 1] (M)

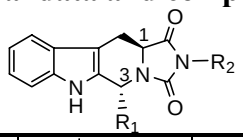

120
100
80
60
40
20
0
% inhibition
0
5
10
15
20
25
30
[compound 6] (M)

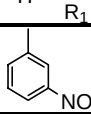
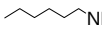
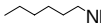
Characterization data

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-(*tert*-butoxycarbonyl)-N_b-(*Z*-2'-iodo-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-(*tert*-butoxycarbonyl)-N_b-(*Z*-2'-iodo-2'-butenyl) L-tryptophan and 5-oxo-2, 2-*bis*(phenylthio)-pentanoic acid methyl ester ($R^{1'}$). 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 [a]



R_1 / R_2												
	Calcd. Mass	Found Mass M-1	Calcd. Mass	Found Mass M-1	Calcd. Mass	Found Mass M-1	Calcd. Mass	Found Mass M-1	Calcd. Mass	Found Mass M-1	Calcd. Mass	Found Mass M-1
	375.16	374.19 (**A1**)	359.16	358.25 (**A2**)	404.15	403.24 (**A3**)	365.21	364.29 (**A4**)	353.21	352.34 (**A5**)	360.16	359.31 (**A6**)
	373.14	372.24 (**B1**)	357.15	356.26 (**B2**)	402.13	401.25 (**B3**)	363.19	362.30 (**B4**)	351.19	350.34 (**B5**)	358.14	357.31 (**B6**)
	427.13	426.18 (**C1**)	411.14	410.25 (**C2**)	456.12	455.25 (**C3**)	417.19	416.29 (**C4**)	405.19	404.23 (**C5**)	412.13	411.24 (**C6**)
	518.25	517.34 (**D1**)	502.26	501.34 (**D2**)	547.24	546.34 (**D3**)	508.30	507.44 (**D4**)	496.42	495.42 (**D5**)	503.25	502.34 (**D6**)
	433.15	432.22 (**E1**)	417.17	416.29 (**E2**)	462.15	461.23 (**E3**)	422.23	421.34 (**E4**)	411.22	410.32 (**E5**)	418.16	417.29 (**E6**)
	418.20	417.33 (**F1**)	402.21	401.45 (**F2**)	447.19	446.28 (**F3**)	408.25	407.34 (**F4**)	396.25	395.29 (**F5**)	403.20	402.36 (**F6**)

[a]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)

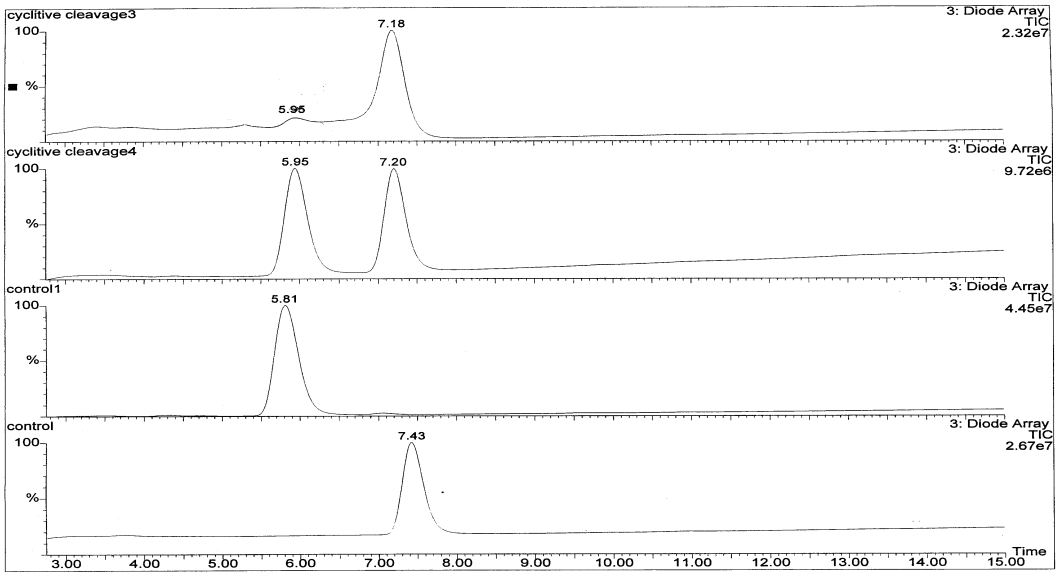
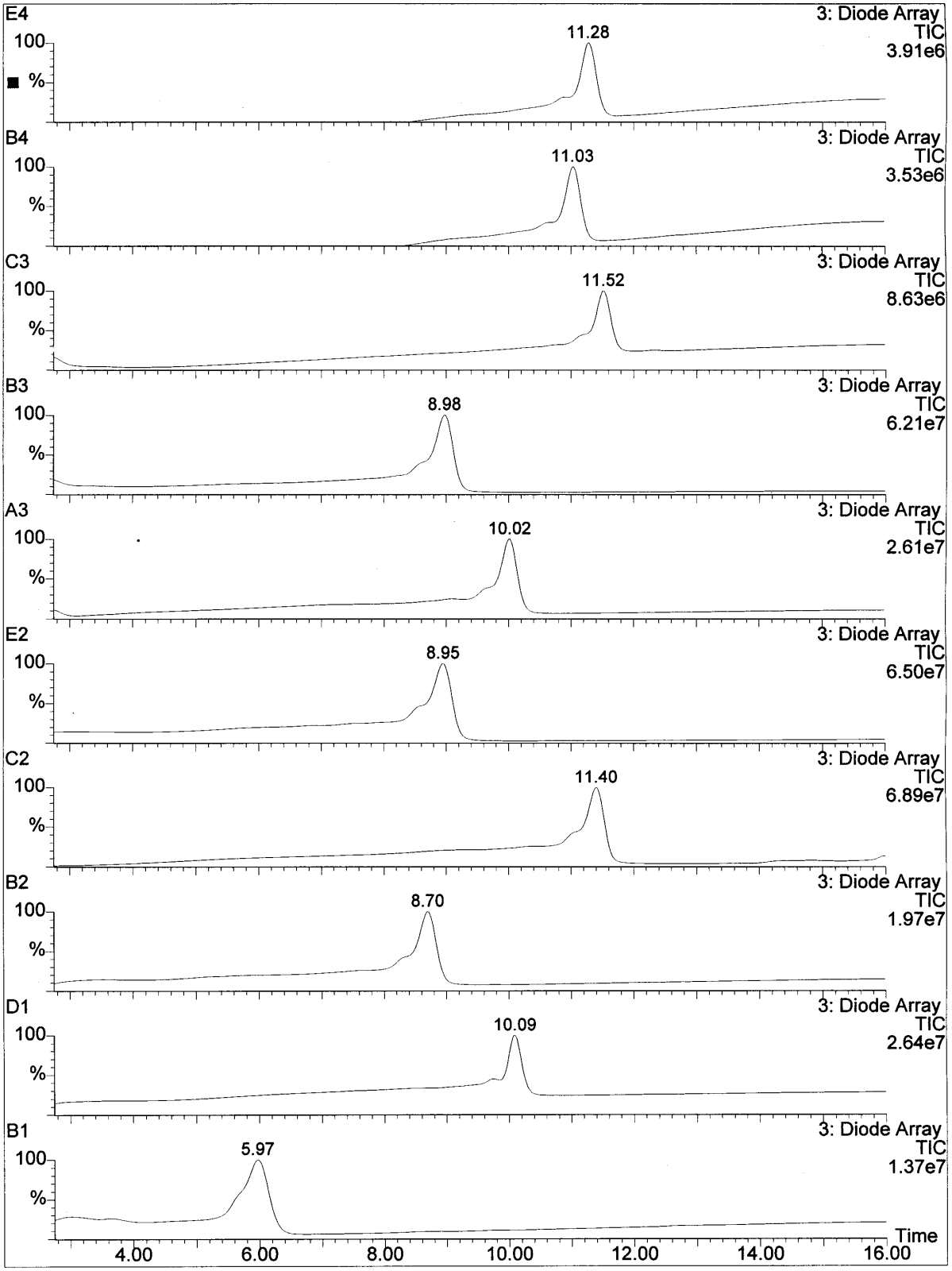


Figure 3. Representative LC traces for compounds synthesized on solid-phase



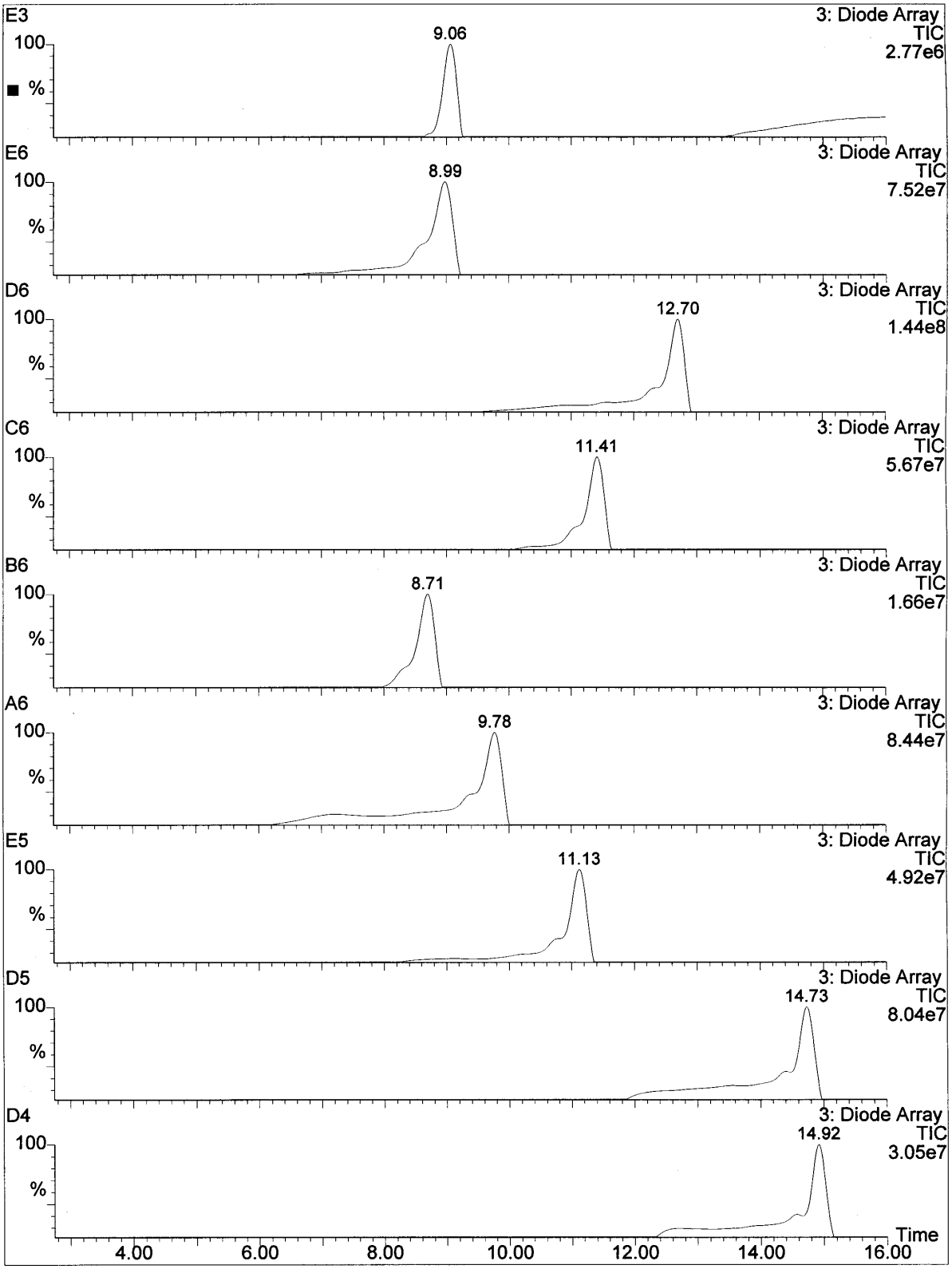
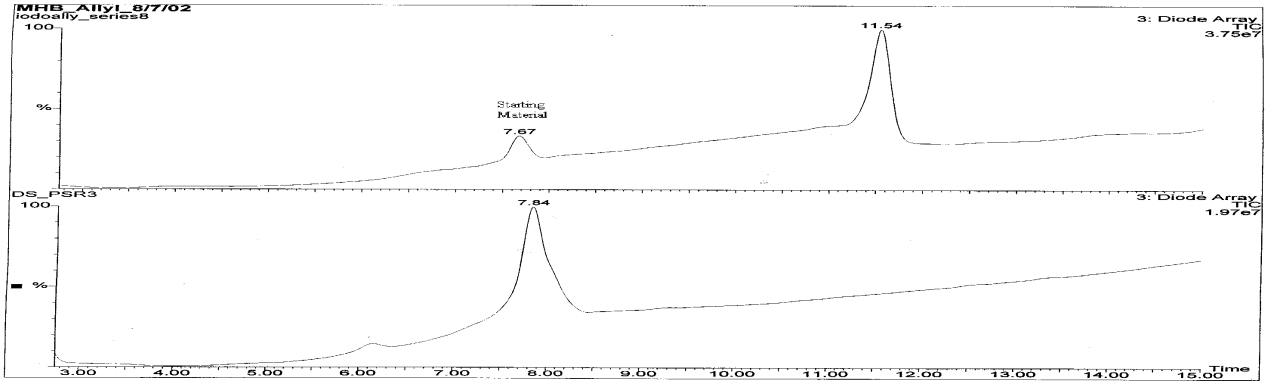
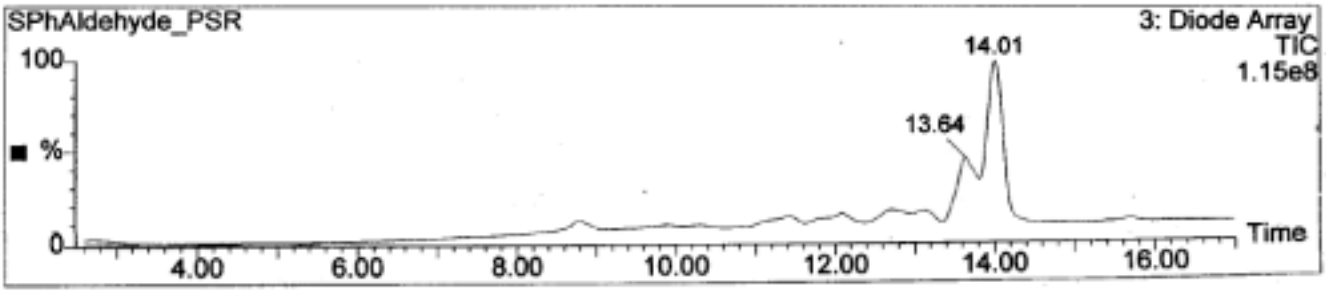

E3
3: Diode Array
TIC
2.77e6
9.06
100
%
E6
3: Diode Array
TIC
7.52e7
8.99
D6
3: Diode Array
TIC
1.44e8
12.70
C6
3: Diode Array
TIC
5.67e7
11.41
B6
3: Diode Array
TIC
1.66e7
8.71
A6
3: Diode Array
TIC
8.44e7
9.78
E5
3: Diode Array
TIC
4.92e7
11.13
D5
3: Diode Array
TIC
8.04e7
14.73
D4
3: Diode Array
TIC
3.05e7
14.92
Time
4.00
6.00
8.00
10.00
12.00
14.00
16.00

Figure 4. LC trace* of cleavage product from the Pictet-Spengler reaction on tenta gel 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)

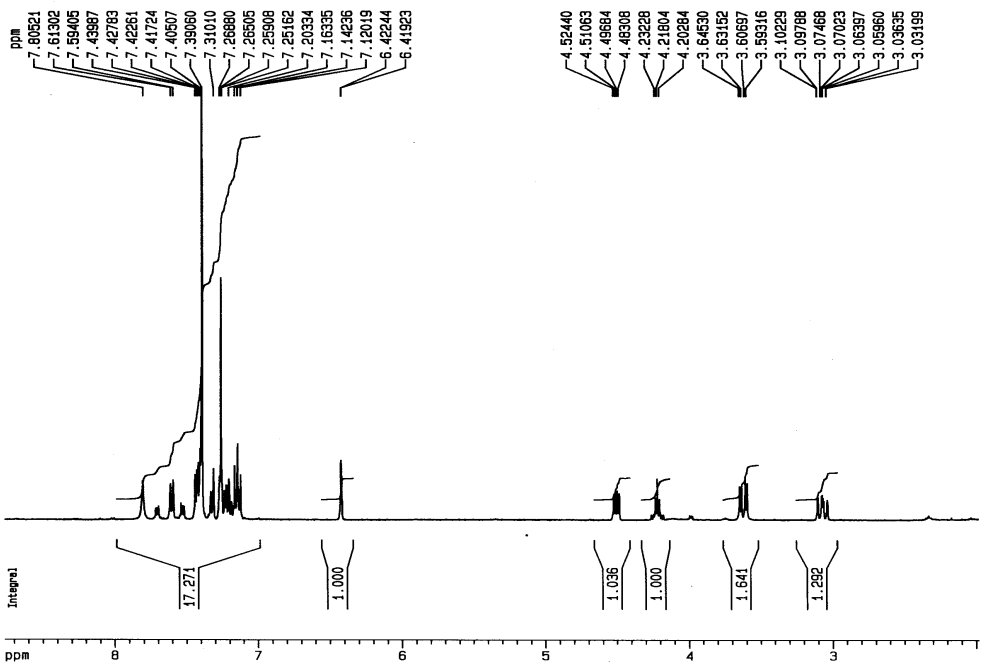
Figure 5. LC trace* of cleavage product from the Pictet-Spengler reaction on Wang resin bound N_b-(*tert*-butoxycarbonyl)-N_b-(Z-2'-iodo-2'-butenyl) L-tryptophan and 5-oxo-2, 2-bis(phenylthio)-pentanoic acid methyl ester



*-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

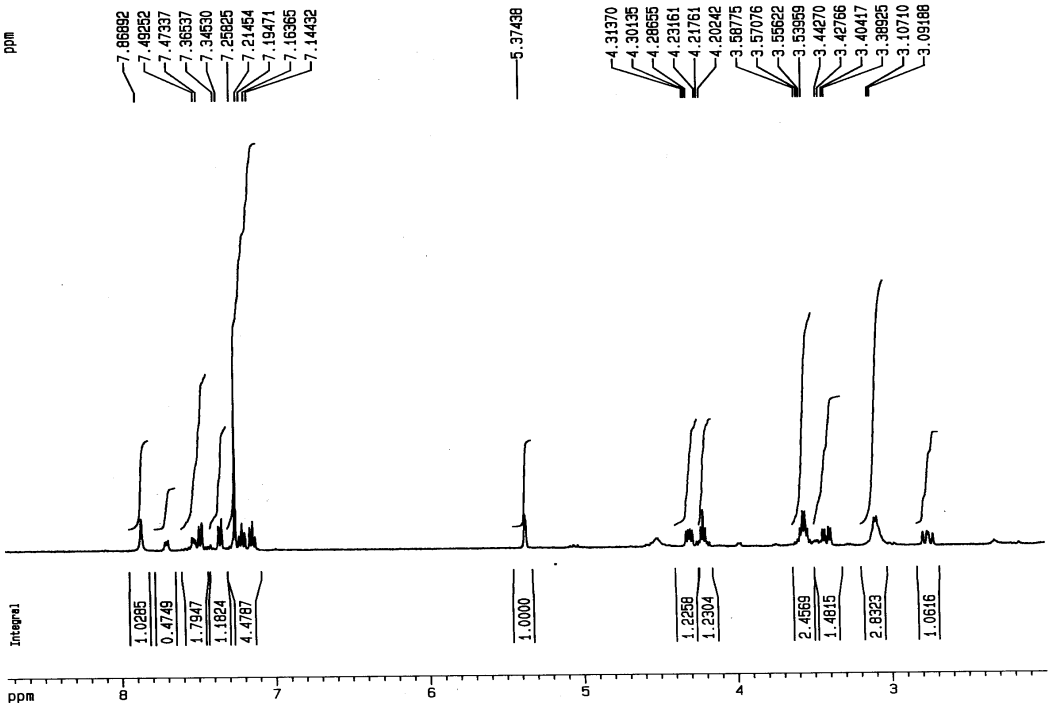
NMR Spectra

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



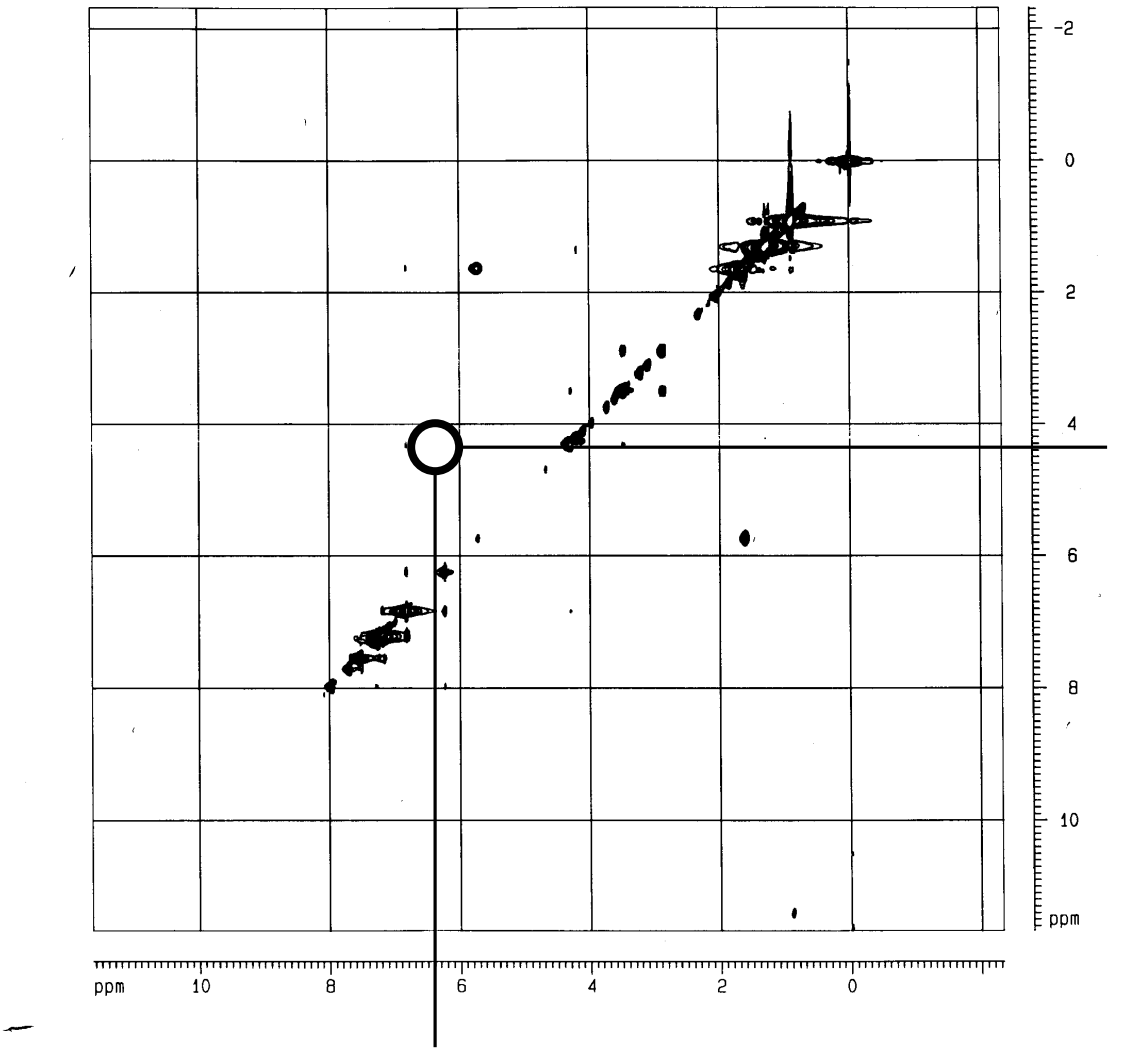

```
Current Data Parameters
NAME                  c2
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20020924
Time               11.56
INSTRUM          dpx400
PROBHD   5 mm Multinucl
PULPROG                zg
TD                 16384
SOLVENT            CDCl3
NS                    32
DS                     4
SWH             5995.204 Hz
FIDRES          0.365918 Hz
AQ              1.3664756 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.0 K
D1             2.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                  8.20 usec
PL1                -2.00 dB
SFO1          400.1318806 MHz

F2 - Processing parameters
SI                 32768
SF            400.1300095 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 20.00 cm
CY                 12.50 cm
F1P                8.767 ppm
F1               3507.93 Hz
F2P                1.981 ppm
F2                792.76 Hz
PPMCM          0.33929 ppm/cm
HZCM          135.75864 Hz/cm
```

Figure 7. ^{1}H-NMR spectrum of library member D5



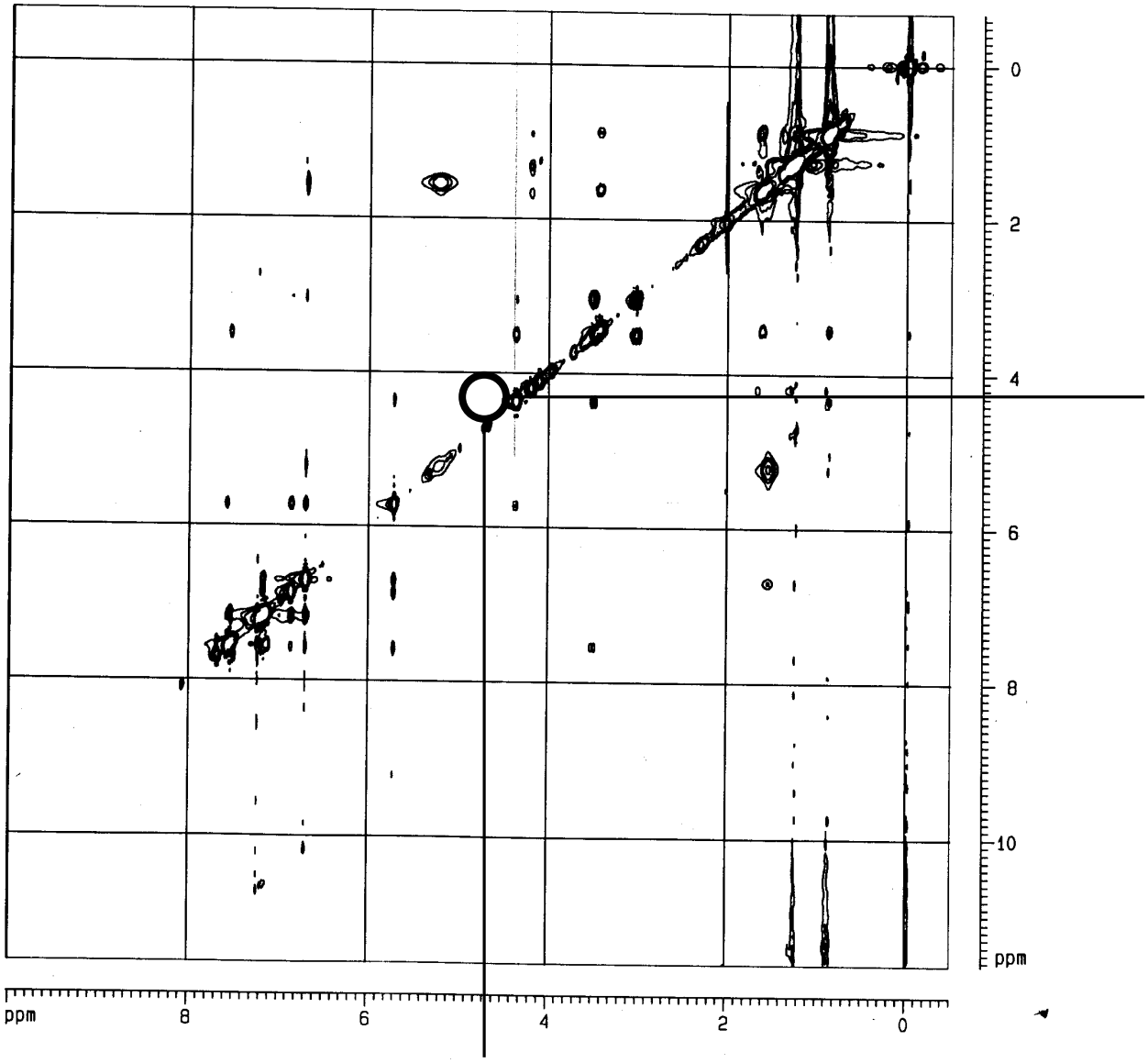

```
Current Data Parameters
NAME                  d5
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20020924
Time               19.32
INSTRUM          dpx400
PROBHD   5 mm Multinucl
PULPROG                zg
TD                 16384
SOLVENT            CDCl3
NS                    32
DS                     4
SWH             5995.204 Hz
FIDRES          0.365918 Hz
AQ              1.3664756 sec
RG                   256
DW                83.400 usec
DE                 6.00 usec
TE                298.0 K
D1             2.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                  8.20 usec
PL1                -2.00 dB
SFO1          400.1318806 MHz

F2 - Processing parameters
SI                 32768
SF            400.1300095 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 20.00 cm
CY                 12.50 cm
F1P                8.750 ppm
F1               3501.14 Hz
F2P                2.000 ppm
F2                800.26 Hz
PPMCM          0.33750 ppm/cm
HZCM          135.04388 Hz/cm
```

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 (δ_H 5.77 ppm) and H-3 (δ_H 4.42 ppm)

References

[1] V. H. Rawal, C. Michoud, R. Monested, *J. Am. Chem. Soc.* **1993**, *115*, 3030-3031.

[2] a) H. R. Snyder, C. H. Hansch, L. Katz, S. M. Parmerter, E. C. Spaeth, *J. Am. Chem. Soc.* **1948**, *70*, 219-221; b) M. F. Brana, M. Garrido, M. L. Lopez, P. de Miguel, A. Riano, *Syn. Commun.* **1990**, *20*, 1793-1810.

[3] G. Massiot, T. Mulamba, *J. Chem. Soc., Chem. Commun.* **1983**, 1147-1149.

[4] F. Garro-Helion, A. Merzouk, F. Guibé, *J. Org. Chem.* **1993**, *58*, 6109-6113.

[5] Isocyanates that are not commercially available are made by either (a) J. H. Tsai, L. R. Takaoka, N. A. Powell, J. S. Nowick, In *Org Syn 78*, 220-222. or (b) K. Ninomiya, T. Shioiri, S. Yamada, *Tetrahedron* **1974**, *30*, 2151-2157.

[6] T. M. Kapoor, T. U. Mayer, M. L. Coughlin, T. J. Mitchison, *J. Cell Biol.* **2000**, *150*, 975-978.

[7] T. M. Kapoor, T. J. Mitchison, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 9106-9111.