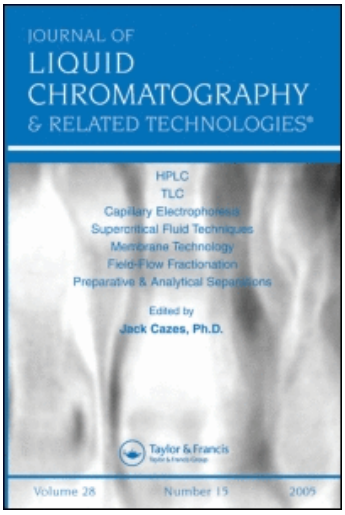


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ENANTIOMERIC SEPARATION OF A SERIES OF 1,4-BENZODIAZEPIN-2-ONE CCK_B RECEPTOR ANTAGONISTS BEARING ACIDIC SUBSTITUENTS BY CHIRAL HPLC

A. P. Watt[a]; D. Rathbone[a]; H. M. Verrier[a]; M. S. Chambers[a]; S. C. Hobbs[a]
[a] Department of Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, U. K.

Online publication date: 22 February 1999

To cite this Article Watt, A. P. , Rathbone, D. , Verrier, H. M. , Chambers, M. S. and Hobbs, S. C.(1999) 'ENANTIOMERIC SEPARATION OF A SERIES OF 1,4-BENZODIAZEPIN-2-ONE CCK_B RECEPTOR ANTAGONISTS BEARING ACIDIC SUBSTITUENTS BY CHIRAL HPLC', Journal of Liquid Chromatography & Related Technologies, 22: 3, 333 — 344
To link to this Article: DOI: 10.1081/JLC-100101663
URL: http://dx.doi.org/10.1081/JLC-100101663

J. LIQ. CHROM. & REL. TECHNOL., 22(3), 333–344 (1999)

ENANTIOMERIC SEPARATION OF A SERIES OF 1,4-BENZODIAZEPIN-2-ONE CCK_B RECEPTOR ANTAGONISTS BEARING ACIDIC SUBSTITUENTS BY CHIRAL HPLC

A. P. Watt,* D. Rathbone, H. M. Verrier,
M. S. Chambers, S. C. Hobbs

Department of Medicinal Chemistry
Merck Sharp and Dohme Research Laboratories
Neuroscience Research Centre
Terlings Park, Eastwick Road
Harlow, Essex, CM20 2QR, U. K.

ABSTRACT

A series of 1,4-benzodiazepin-2-one CCK_B receptor antagonists are reported in which substitution at C3 of the benzodiazepine by a phenyl-urea group bearing acidic moieties has generated a chiral centre. As one of these enantiomers is substantially more selective for the CCK_B over the CCK_A receptor, an analytical separation of the enantiomers was developed to monitor the resolution of compounds by chemical means. It was shown that such compounds may be resolved using a Pirkle-type 3,5-dinitrobenzoyl-leucine chiral stationary phase to give high α and R_S values. However, traditional mobile phase methodologies proved unsuccessful with these compounds which were found not to elute without the addition of acetic acid. An investigation is described in which the effect of the acidic substituent, mobile phase composition, including addition of acid, and temperature is discussed for such compounds.

INTRODUCTION

Cholecystokinin (CCK) is a 33 amino acid polypeptide hormone which is found in both the gastrointestinal tract and the central nervous system. There are two distinct CCK receptor subtypes which are designated CCK_A and CCK_B, and action at these receptors has been implicated in a number of physiological processes.

For example, the CCK_B receptor, which is found predominantly in the gut, is thought to play a role in pancreatic secretion, gut motility and gall bladder contraction.[1,2] The CCK_B receptor is found primarily in the brain and is closely related to the stomach gastrin receptor.[3] It is postulated that the CCK_B receptor has a neuromodulatory role in satiety and anxiety.[4]

In an attempt to elucidate further the physiological significance of these receptors, non-peptidic CCK_B receptor antagonists have been extensively researched and developed[5] including a series of 1,4-benzodiazepin-2-ones.[6-7]

It was demonstrated that the stereochemistry at C3 of the benzodiazepine ring was important for receptor subtype selectivity[8] with the 3*S* enantiomer generally being more selective for CCK_A and the 3*R* enantiomer more selective for CCK_B.

Further modifications of the 1,4-benzodiazepin-2-one skeleton to improve affinity and selectivity lead to the discovery of a compound in which C5 phenyl is replaced by cyclohexyl.[10] In an effort to improve the solubility of this molecule whilst retaining or improving affinity and selectivity, a series of compounds bearing cationic substituents[11] and acidic substituents on the phenylurea were prepared.[12]

As this whole series bearing acidic substituents was to be resolved to prepare the preferred 3*R* enantiomer, a chromatographic method for the monitoring of enantiomeric purity was required. A system employing a Pirkle DNBL column was envisaged as separation of 1,4-benzodiazepine-2-ones using Pirkle type DNBPG or DNBL chiral stationary phases has already well been documented where the C5 substituent was phenyl[9] or cycloalkylamine.[13]

A model compound was chosen to study the effects of mobile phase composition and temperature in order to ensure operation at or near the optimum conditions and these experiments are described.

Additionally, it was found that the behaviour of acidic compounds on the Pirkle columns was very different to that encountered previously and the work done to optimize this is also shown.

EXPERIMENTAL

Materials

All compounds described were synthesized in-house with identity and purity confirmed by NMR, MS, HPLC, and elemental analysis. HPLC grade methanol, 1-chlorobutane and glacial acetic acid (AR grade) were obtained from Fisons (Loughborough, UK).

Instrumentation

An HP1090L series high performance liquid chromatograph was used for the analytical separations (Hewlett Packard, Avondale, USA). The system comprises of an autoinjector, consisting of a Rheodyne 7010 injection valve fitted with a fixed 250µL loop, an autosampler, and a binary DR-5 solvent delivery system. Detection was by UV using a built-in filter photometric detector (FPD) and data was processed using an HP DOS ChemStation. Column temperature was regulated using a Violet T-55S column cooler (Flowgen, UK).

Chromatographic Conditions

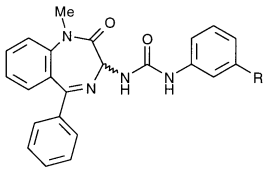
HPLC analysis was performed using columns containing 3,5-(dinitrobenzoyl)-leucine (DNBL) covalently bonded to silica, supplied by Hichrom (Reading, UK). The dimensions were 250 x 4.6mm i.d. with a silica particle size of 5µm. Typical mobile phases were 5% MeOH in 1-chlorobutane. The flow rate analytically was 1.0 mL/min. The FPD was set to 230nm, this being the approximate λ_{max} for these compounds. 5µL of a 1mg/mL solution of all compounds were injected for the analytical analyses which were performed at thermostatically regulated temperature.

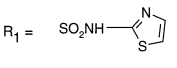
RESULTS AND DISCUSSION

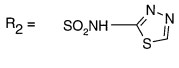
The range of substituents analyzed is shown (Table 1) and can be sub-divided into three series; the acyl suphonamides (compounds 1-5), the reverse acyl suphonamides (compounds 6-10) and carboxylate and bioisosteres (compounds 11-13). Initial analyses for these compounds were performed using MeOH in 1-chlorobutane mobile phase mixtures as this had been shown to confer advantages in terms of peak shape over hexane/ethanol mixtures for such compounds.[13] It was noted that, using a brand new column, retention was adequate although slightly longer than anticipated based on previous experience,

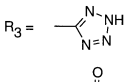
Table 1

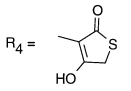
Variation of Capacity Factors, Selectivity, and Resolution with Acidic Substituent*











ID	Substituent	k'$_1$	k'$_2$	α	R_S
1	$CONHSO_2iPr$	0.58	1.06	1.85	3.50
2	$CONHSO_2Et$	1.06	1.92	1.82	4.07
3	$CONHSO_2Ph$	2.03	3.42	1.68	3.82
4	$CONHSO_2Me$	2.57	4.70	1.83	4.77
5	$CONHSO_2CF_3$	>44	>44		
6	$SO_2NHCOiPr$	0.17	0.36	2.11	2.69
7	$SO_2NHCOMe$	1.48	1.02	2.10	4.06
8	$SO_2NHCOPh$	3.13	5.34	1.71	4.29
9	R_1	0.51	1.05	2.04	4.19
10	R_2	3.66	7.31	2.00	1.67
11	COOH	2.42	4.85	2.01	4.95
12	R_3	6.84	12.20	1.79	3.48
13	R_4	20.60	32.20	1.57	2.55

* HPLC conditions: Pirkle DNBL column (250 x 4.6mm i.d.); Mobile Phase 20% MeOH in 1-chlorobutane (+0.5% HOAc); Flow rate 1 mL min^{-1}; Detection UV @ 254 nm; Loading 5μg per injection performed at ambient temperature.

but on repeated re-injection retention became increasingly lengthened until no elution at all was observed. However, on washing the column with ten volumes of hexane containing 0.1% trifluoroacetic acid and then repeating the analysis, initial column performance appeared to be restored.

This suggested that the compounds were binding strongly to the column by virtue of their polar acidic substituents interacting with exposed silanol groups in a non-specific fashion possibly by hydrogen-bonding. However, by washing with an acid containing mobile phase, such adsorption sites are saturated and normal column performance could be returned. Furthermore, as the acid washes

off over time, so binding sites on the column again become exposed and retention increases. An obvious solution to this then is to include an acid in the mobile phase to provide a constant background of H^+ and so stabilize performance. As it is known that strongly acidic conditions will cause a reduction in column life for silica-based stationary phases, acetic acid was preferred over TFA.

Using the phenyl acyl sulphonamide 3 as a model compound, the requirement for acetic acid in the mobile phase was investigated. As the changes in capacity factor were found to be strongly dependent on equilibration times, an investigation into this suggested that at least 1 hour was required for the retention to fully stabilize after changing the proportion of acid hence this was adhered to throughout the experiment. Using 1% acetic acid, good chromatography is observed but it is noted that capacity factors are seen to increase as the amount of acid is decreased. This is represented graphically by considering the effect on both the capacity factors (Figure 1a), separation selectivity α (Figure 1b), and resolution R_S (Figure 1c).

The inclusion of acetic acid in the mobile phase could be considered as a use of the so-called "polar-organic mode" in which an acetonitrile mobile phase is used with a cyclodextrin-based stationary phase.[14-16] In this, in order to elicit enantioselectivity, a small amount of methanol as an H-bonding modifier is included with varying proportions of acetic acid and triethylamine. In this mode it is clear that the addition of the acid and base is crucial to the chiral recognition process as without it enantioselectivity is greatly diminished or lost.[14] The suggestion is that the degree of protonation of ionizable functionality in the analyte will be affected by the relative proportions of acid and base and this will in turn affect the nature of the interaction with the CSP.

In contrast, in this system, there is only a slight trend in both the separation selectivity (virtually unchanged with increasing acid) and resolution (which decreases with increasing acid) suggesting that the acid does not participate significantly in any stereospecific retention mechanism. This would be entirely consistent with both an interaction of the solute with the silica surface rather than the chiral selector which can be masked by the addition of acid and with the ionizable functionality being remote from the chiral centre and therefore not participating in the chiral selectivity.

Resolution shows a small downward trend with increasing acid concentration; since the separation selectivity remains unchanged this suggests that the change in mobile phase composition may marginally affect peak shape, although this is by no means significant.

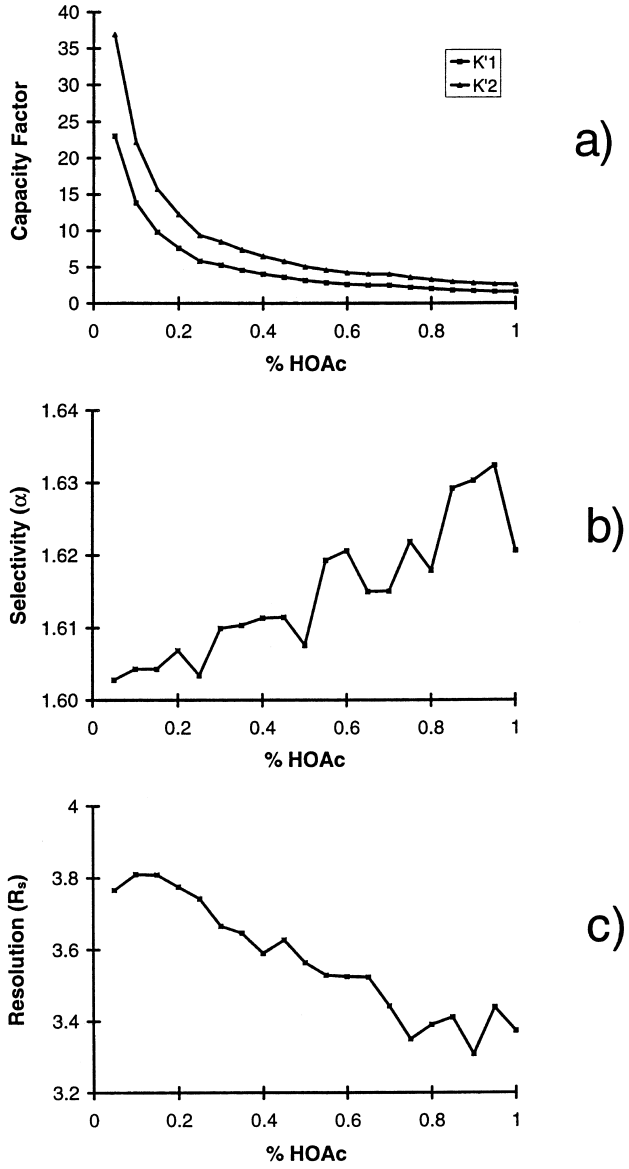


Figure 1. Variation of a) capacity factor, b) separation selectivity, and c) resolution of compound 3 with % HOAc from 0.05 to 1%; HPLC conditions as before.

Although the addition of a base such as triethylamine was not attempted, comparisons with polar-organic separations may be misleading. It is more likely that a mode of separation following the classical Pirkle model is being demonstrated as for other related benzodiazepines ;[13] indeed the elution order found for these compounds is always 3*R* before 3*S* which strongly supports this.

Using identical conditions of 20% MeOH in 1-chlorobutane with 0.5% HOAc, the three series of compounds were analyzed and the separation selectivities and resolution calculated (Table 1). In the first series, with a substituted acyl sulphonamide, it can be seen that although decreasing lipophilicity from iPr to Et to Me does result in a decrease in retention, the capacity factors of the trifluoromethyl analogue appear significantly longer than would be anticipated by its lipophilicity. This suggests that the acidity of the substituent may play a role in the retention characteristics of these compounds as the electron-withdrawing effect of the CF_3 group would make this analogue considerably more acidic than the others. The separation selectivity of the phenyl analogue was lower than observed for the other analogues; however, resolution was maintained. It would appear, therefore, that either H-bonding or ъ-ъ interactions are significant in contributing towards the capacity factors but actually appear to have little bearing on the chiral recognition process.

In the second group, the reverse acyl sulphonamides, the phenyl analogue appeared to be an interesting compound with considerably higher capacity factors than the other compounds, perhaps suggesting that the benzoyl group may be participating in the retention mechanism with the CSP. This interaction is beneficial for the chromatography in terms of peak shape although this is not a chiral interaction as the separation selectivity is decreased but the resolution actually improved over the other derivatives.

The third group of carboxylic acid and various replacements again illustrates the role of H-bonding in controlling retention with increased H-bonding potential resulting in larger capacity factors. Although, therefore, a great deal of difference in these compounds can be observed in terms of the capacity factors, the chiral recognition remains essentially unaffected. This can be attributed to the dominant interaction being the ъ-acid/ ъ-base interaction of the benzodiazepine moiety and the dinitrobenzoyl group of the CSP. Chiral recognition is then attained through the interactions resulting from the directionality of groups such as the carbonyl at C2 and the urea at C3. The acid group, being remote from this centre can therefore affect the overall retention characteristics but not the chiral interaction as this is likely to be oriented away from the CSP backbone.

In investigating the optimal mobile phase conditions further, the proportion of acetic acid was held constant at 0.5%, giving adequate capacity factors of less than ten for both enantiomers and the percentage of methanol in 1-chlorobutane

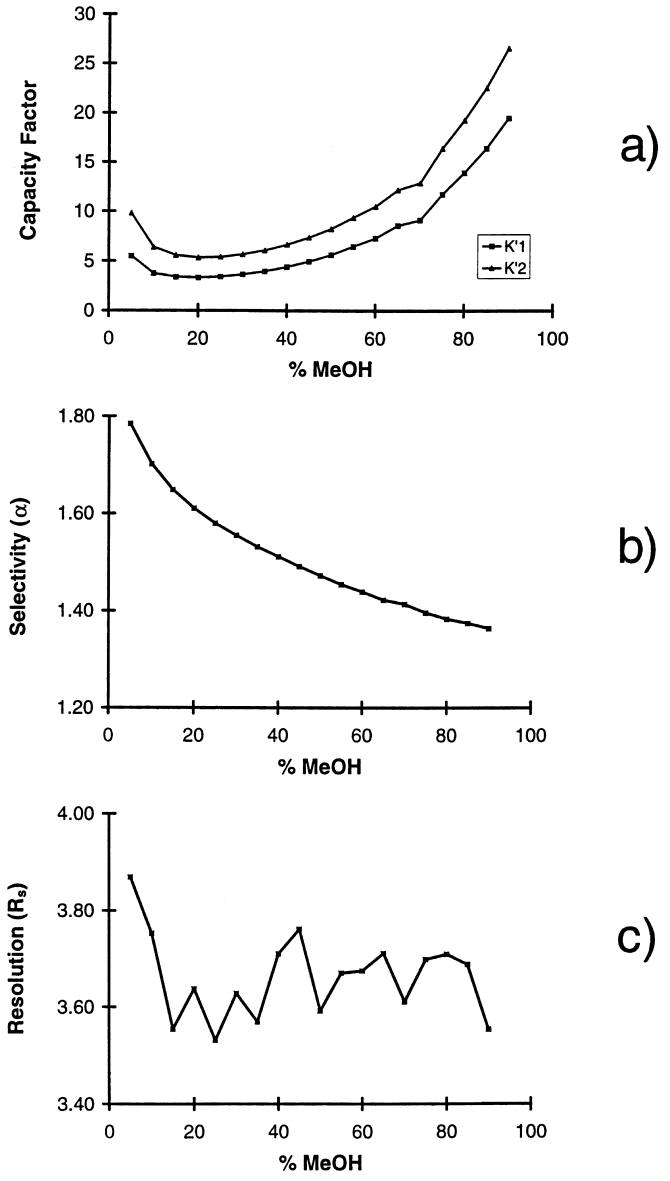


Figure 2. Variation of a) capacity factor, b) separation selectivity, and c) resolution of compound 3 with % MeOH from 5 to 90%. The concentration of HOAc was held constant at 0.5%, other HPLC conditions as before.

was varied from 0% to 90% in 5% increments, again using 3 as the model compound. From the plot of capacity factor vs. % MeOH (Figure 2a), it can be seen that the graph is parabolic, with a minimum for the capacity factors at approximately 20% MeOH. At concentrations of less than 5% MeOH elution takes more than 200 minutes but decreases with increasing MeOH up to 20%. This suggests that without MeOH the analyte adsorbs strongly to the column through H-bonding and addition of MeOH disrupts this H-bonding resulting in elution.

From 20% to 90% MeOH, a general increase in capacity factor is observed for both enantiomers although the separation selectivity decreases (Figure 2b) and resolution remains unaffected (Figure 2c). This would not be the behaviour anticipated if the column was underivatised silica, hence the Pirkle substituent is clearly contributing significantly to the retention. The interaction between the mobile and stationary phase is obviously complex with many competing factors but it can be seen that no advantage is to be gained by working significantly away from 20% MeOH.

The column temperature was varied from 0°C to 50°C in 5°C increments in order to establish the effect of working at extremes away from room temperature. Capacity factors for both enantiomers are seen to decrease with increasing temperature (Figure 3a). Additionally, the separation selectivity is seen to decrease with increasing temperature (Figure 3b) but resolution increases (Figure 3c). This is what would be predicted as the degrees of freedom of both the CSP and the analyte will increase with increasing temperature thereby decreasing the energy of interaction for both enantiomers. The second eluting enantiomer appears to be proportionately more affected than the first eluting one, suggesting that it is the interactions specific to enantiomer 2 that are being disrupted so decreasing the separation selectivity.

One possible explanation for the increase in resolution could be that the increase in temperature gives rise to a decrease in mobile phase viscosity. The improved mass-transfer which will result from this decreased viscosity should lead to a decrease in peak-width and hence an improvement in resolution.

CONCLUSIONS

The separation, as designated by the separation selectivity α, and resolution R_s of the CCK_B antagonists discussed are, in general, not dependent on the nature of the acidic substituent using a Pirkle-type DNBL CSP. However, the nature of the substituent and in particular its H-bonding potential appears to have a profound effect on the capacity factors.

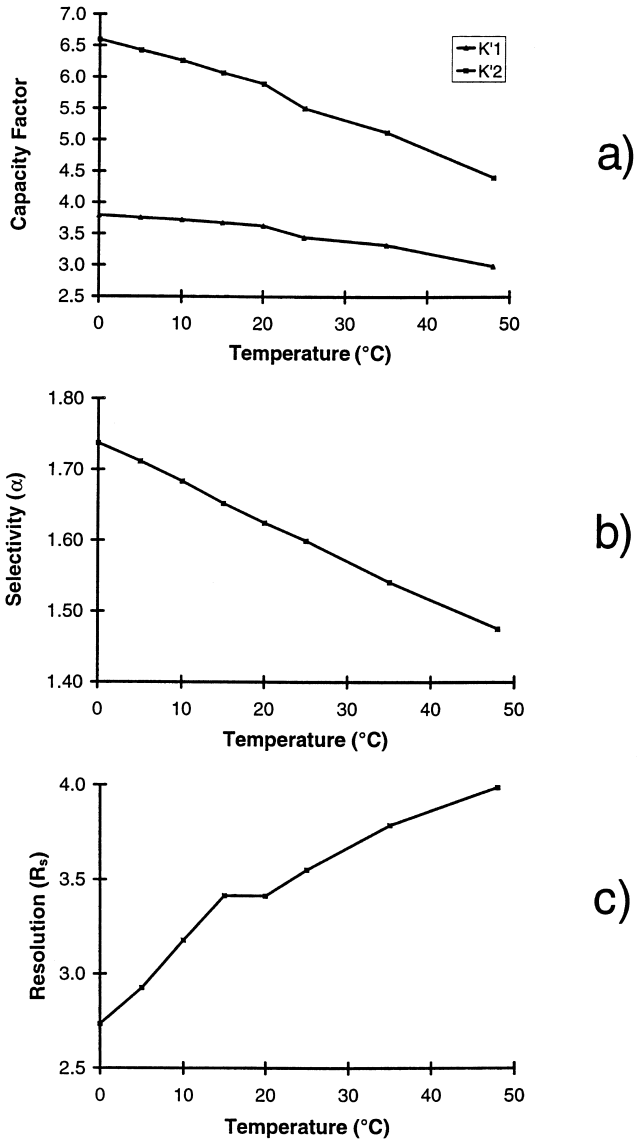


Figure 3. Variation of a) capacity factor, b) separation selectivity, and c) resolution of compound 3 with temperature from 0 to 50°C; HPLC conditions as before.

In summary, with the exception of 5, all compounds were found to chromatograph adequately to allow either analytical determination of chiral purity or scale-up to preparative chromatography to resolve racemic material. The crucial finding, both analytically and preparatively was the requirement for a competing acid, in these examples acetic acid, to be added to the mobile phase at concentrations up to 1% in order to maintain reproducible column performance, particularly in terms of capacity factors.

ACKNOWLEDGMENTS

The authors wish to thank Victor Matassa and Ray Baker for their discussions and support and Desmond O'Connor, Steve Matheson, and Steve Feeley for their expert technical contributions.

REFERENCES

1. R.Y. Wang, Ann. N.Y. Acad. Sci., **537**, 362-379 (1988).

2. M. Albus, Prog. Neuro-Psychopharmacol. Biol. Psychiatry, **12**, S5-S21 (1988).

3. T. Moran, R.Robinson, M. S. Goldrich, P. McHigh, Brain Res., **365**, 175-179 (1986).

4. R. S. Chang, V. J. Lotti, R. L. Monaghan, J. Birnbaum, E. O. Stapely, M. A. Goetz, G. Albers-Schonberg, A. A. Patchett, J. M. Liesch, O. D. Hensens, J. P. Springer, Science, **230**, 177-179 (1985).

5. M. G. Bock, R. M. DiPardo, B. E. Evans, K. E. Rittle, W. L. Whitter, D. F. Veber, P. S. Anderson, R. M. Friedinger, J. Med. Chem., **32**, 16-23 (1989).

6. R. S. Chang , T. B. Chen, M. G. Bock, R. M. Freidinger, R. Chen, A. Rosegay, V. J. Lotti, Mol Pharmacol, **35**, 803-808 (1989).

7. V. J. Lotti, R. S. Chang, Eur. J. Pharmacol., **162**, 273-280 (1989).

8. M. G. Bock, Drugs Fut., **16**, 631 (1991).

9. W. H. Pirkle, A. Tsipouras, J. Chromatogr., **291**, 291-298 (1984).

10. M. S. Chambers, S. C. Hobbs, S. R. Fletcher, V. G. Matassa, P. J. Mitchell, A. P. Watt, R. Baker, S. B. Freedman, S. Patel, A. J. Smith, Bioorg Med. Chem. Lett., **3(10)**, 1919-1924 (1993).

11. G. A. Showell, S. Bourrain, J. G. Neduvelil, S. R. Fletcher, R. Baker, A. P. Watt, A. E. Fletcher, S. B. Freedman, J. A. Kemp, G. R. Marshall, S. Patel, A. J. Smith, V. G. Matassa, J. Med. Chem., **37**, 719-721 (1994).

12. M. S. Chambers, S. C. Hobbs, M. I. Graham, A. P. Watt, S. R. Fletcher, R. Baker, S. B. Freedman, S. Patel, A. J. Smith, V. G. Matassa, Bioorg. Med. Chem. Lett., **5(20)**, 2303-2308 (1995).

13. A. P. Watt, H. M. Verrier, D. Rathbone, J. Liq Chrom. & Rel. Technol., **20(1)**, 111-121 (1997).

14. D. W. Armstrong, S. Chen, D. Chang, S. Chang, J. Liq. Chromatogr., **15(3)**, 545-556 (1992).

15. S. C. Chang, G. L. Reid III, S. Chen, C. D. Chang, D. W. Armstrong, Trends Anal. Chem., **12(4)**, 144-153 (1993).

16. M. Pawlowska, S. Chen, D. W. Armstrong, J. Chromatogr., **641**, 257-265 (1993).

Received March 15, 1998
Accepted June 16, 1998
Manuscript 4774

Request Permission or Order Reprints Instantly!