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Development of analytical and preparative chromatographic separations of novel growth hormone secretagogue compounds

Joseph H. Kennedy*, John L. Bowers, Jeffrey A. Dodge, Charles W. Lugar, Timothy A. Shepherd, V. Scott Sharp

Chemical Process Research and Development, Lilly Research Laboratories, A Division of Eli Lilly Company, 1200 Kentucky Avenue, Indianapolis, IN 46060, USA

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Abstract

Chromatographic separations of new growth hormone secretagogue compounds were developed to support structure–activity relationship (SAR) studies in conjunction with lead optimization. These new compounds differed from Merck's MK-677 by having two chiral centers and thus diastereomeric mixtures were generated. Separation of initial compounds in the SAR was achieved on a Kromasil C_{18} column using an ammonium acetate buffer and acetonitrile. However, additional candidates were not separable on C_{18} columns and a chiral Kromasil CHI-DMB column was used to resolve the diastereomeric compounds. The Kromasil CHI-DMB packing was also used in a preparative chromatographic system to resolve multigram quantities of secretagogue candidates for testing. Chiral separations of different intermediates were also developed in support of evolution of an asymmetric synthetic route. This report summarizes development of the preparative chromatographic system used to purify diastereomeric mixtures and chiral separations of intermediates in the synthesis.

Keywords: Preparative chromatography; Growth hormone secretagogue

1. Introduction

Since initial discovery in the 1970s that small peptides cause the release of growth hormone (GH) from the pituitary, considerable attention has been directed toward identifying peptidomimetrics with improved oral bioavailability. This has resulted in identification of various classes of GH secretagogues including benzolactams [1], tripeptides [2], and spiroindanes [3], among others. The most extensively studied oral secretagogue has been MK-0677, illustrated in Fig. 1. Few reports are available on chromatographic separations for these types of molecules. Separation of MK-0677 and another analog (methyl group in place of ether oxygen) has been reported on a C_{18} column using acetonitrile (ACN), water containing 0.1% formic acid, and 10 m*M* ammonium acetate [4]. MK-0677 has only one asymmetric center and thus control of the chemistry yields a single compound when optically pure starting material is used.

Our initial GH secretagogue compounds were diastereomeric with two asymmetric centers. Specific isomers of these compounds were needed for development to continue. With the complexity of these molecules, significant chemical development would

*Corresponding author. Fax: +1-317-276-4507.

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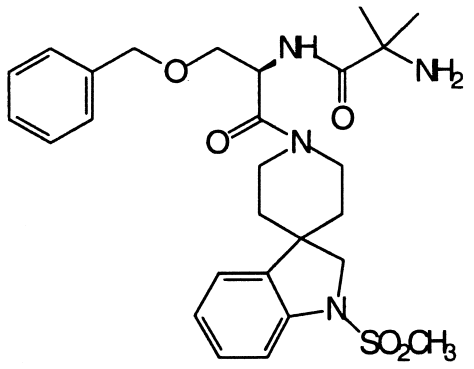


MK-0677

Fig. 1. MK0677.

be necessary to deliver pure diastereomers. This effort would reduce the number of possible candidates in a structure–activity relationship (SAR) study. Development of chromatographic separations and scale-up provided a timely method to deliver quantities of pure compounds from diastereomeric mixtures thus allowing increased synthetic efforts in providing more molecules for the SAR study.

2. Experimental

2.1. Materials

GH secretagogue compounds for the SAR studies were synthesized in Lilly Research Laboratories. Analytical 25×0.46 cm Chiralcel OD and Chiralpak AD columns along with 20 μm bulk Chiralpak AD packing were purchased from Chiral Technologies (Exton, PA, USA). Prepacked 25×0.46 cm Kromasil CHI-DMB and CHI-TBB analytical columns and bulk quantities of 10 μm Kromasil CHI-DMB packing were obtained from Akzo Nobel (Eka Chemicals, Bohus, Sweden). The 2.5 kg Biotage silica cartridge for flash chromatography was purchased from Dyax (Charlottesville, VA, USA). All solvents were HPLC grade. The *n*-heptane used in preparative separations was purchased from Phillips Petroleum (Bartlesville, OK, USA). Bulk ethanol, isopropanol (IPA), methanol (MeOH) and methylene chloride (CH_2Cl_2) were obtained from Superior Solvents and Chemicals (Indianapolis, IN, USA). The *N*,*N*-dimethylethylamine (DMEA) eluent additive was acquired from Aldrich (Milwaukee, WI, USA).

2.2. Instrumentation

All analytical scale separation optimization experiments were performed on Shimadzu HPLC systems (Columbia, MD, USA). Preparative chromatography experiments were completed using a LC-80 automated chromatography system with an 8 cm DAC column purchased from Prochrom (Indianapolis, IN, USA).

Optimization experiments for preparative chromatography were completed on analytical columns and scaled up based on the square of difference in cross sectional area of the columns. Silica gel separation of diastereomers was done via flash chromatography using 30×15 cm cartridges on a Biotage chromatography system.

3. Results and discussion

The basic structure for secretagogue compounds in SAR studies is presented in Fig. 2. Analogs were generated by modification of the various sub-units and data was collected to determine the main sources of activity.

Six compounds (LY359604, LY408117, LY408118, LY408787, LY408650 and LY408642) were evaluated initially. All early compounds had the same benzyl ether substitution on the serine sub-unit. The aryl sub-units were phenyl, naphthyl, *p*-methoxy, biphenyl and *p*-trifluoromethyl substitution. The amide sub-units were 4-methyl piperdine, pyrrolidine and proline methyl ester.

3.1. Development of preparative conditions

LY359604 diastereomers were successfully resolved using an eluent consisting of ACN–water (35:65) containing 0.1% ammonium acetate on a 25×1 cm Kromasil C_{18} column. The flow-rate was 4.0 ml/min. Loading of 1 mg and 3 mg mixtures are illustrated in Fig. 3. Partial separation of the diastereomers of the remaining five compounds was

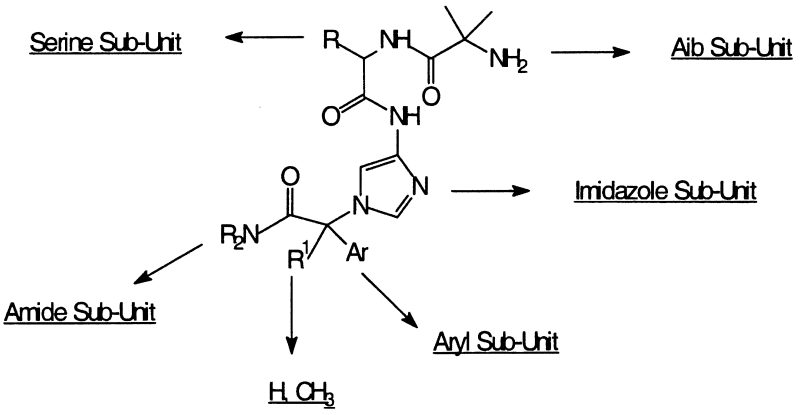


Fig. 2. Secretagogue sub structure.

achieved using a Kromasil C_{18} column and eluent consisting of ACN–water containing 2% ammonium acetate. The use of 0.1% trifluoroacetic acid (TFA) as a modifier in place of ammonium acetate did not improve the separation.

The compounds were examined on a Chiralpak AD column using an IPA–hexane eluent with DMEA as a modifier. The compounds were not resolved and were difficult to elute from the amylose based chiral stationary phase (CSP). Since all compounds were amines, separation was evaluated on *N,N′*-diallyltartardiamide derivative (DATD) modified CSPs from Eka Nobel. The DATD CSPs are chemically bonded and should provide improved stability over traditional coated phases. Basic compounds have previously been resolved on these CSPs [5]. Partial resolution of most of the compounds was obtained on a Kromasil CHI-TBB column using 15% IPA, 5% MeOH, and hexane containing 0.2% DMEA. Baseline resolution was obtained on the Kromasil CHI-DMB using the same eluent. TFA and water were evaluated as additives to improve the peak shape without success. The acidic eluent had a negative affect on peak symmetry.

The limiting factor in most preparative separations is not the isomer separation, but solubility of the analytes in the eluent. GH secretagogue analogs containing an indole function in the serine sub-unit and naphthyl function in the aryl sub-unit were difficult to dissolve at 20 mg/ml and thus loading was reduced for these compounds. The indole/naphthyl compounds also contained higher levels of impurities than other analogs thus making purification more difficult. Modifications of the imidazole sub-unit had some effect on their separation on the Kromasil CSP, but isolations were still obtained. Changes in the amide sub-unit generally had little affect on separation or solubility, except in the case of the pyrazole moiety. Pyrazole analogs were not separable on this CSP. Analytical separation was possible on a Chiralpak AD CSP using an eluent consisting of 50% IPA/heptane and 0.2% DMEA. Scale up of this separation on a 20×8 cm Chiralpak AD was difficult and shave/recycle chromatography was necessary to purify the two diastereomers. In this technique, the front and back of the band are ''shaved'' during the recycle. Pure isomer 1 is collected in front, isomer 2 is collected in back, and the middle is recycled. The cycle can be repeated to increase yield.

3.2. Scale up and optimization

With separation conditions in place, the method was scaled up to handle larger sample loads. A 228-g amount of Kromasil CHI-DMB was slurried in 350 ml of *n*-propanol and packed in a 18×5 cm Prochrom column. The column was equilibrated with an eluent consisting of 5% MeOH, 8% IPA, and heptane containing 0.2% DMEA at a flow-rate of 100 ml/min. Approximately 200 mg of each mixture was dissolved in 15 ml of eluent and pumped onto the 5 cm column. Fractions of isomer 1 and 2 were collected and analyzed. Analyses of the fractions

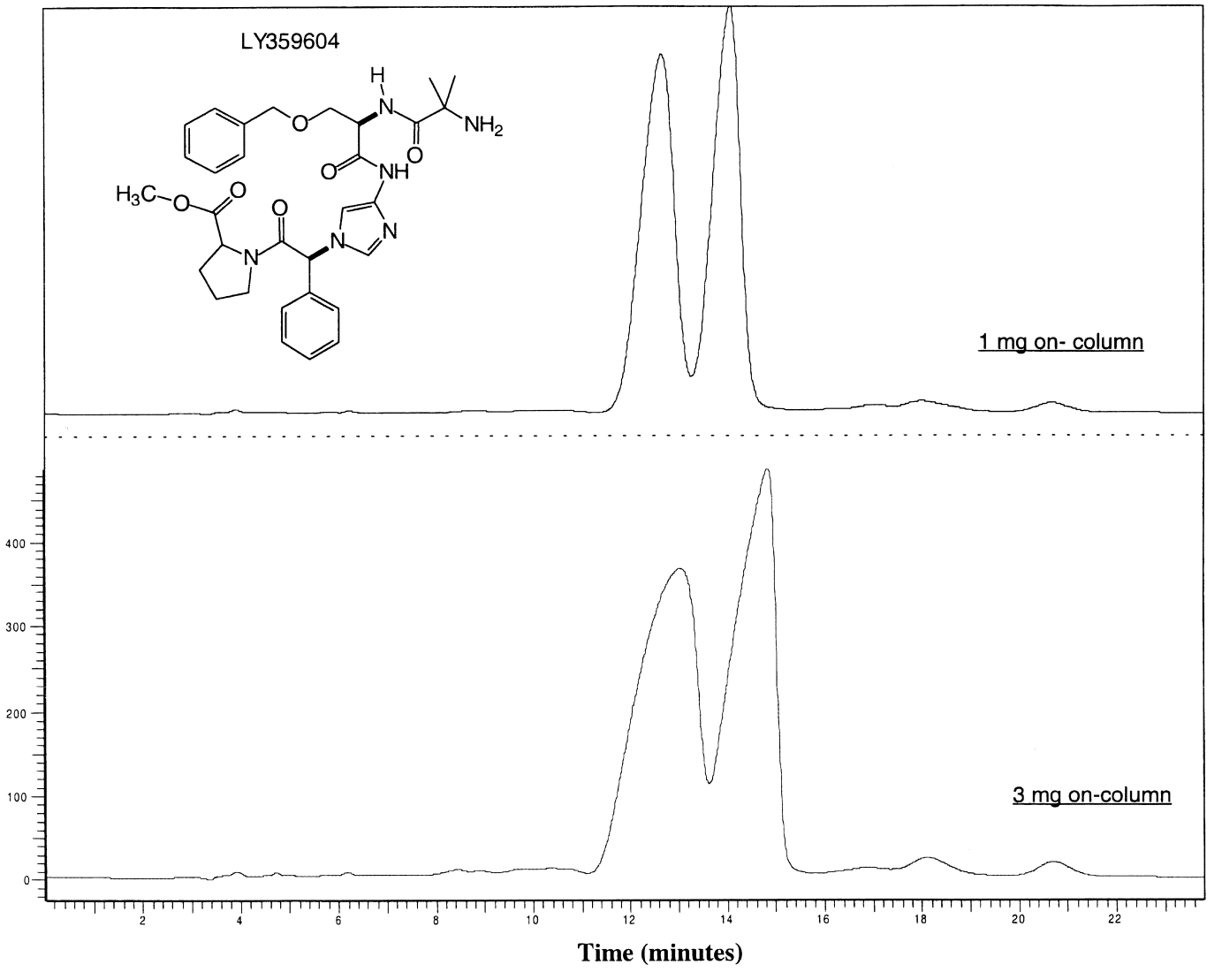


Fig. 3. Separation of diastereomers of LY359604 on a 25×1 cm Kromasil C_{18} column. Conditions as in the text.

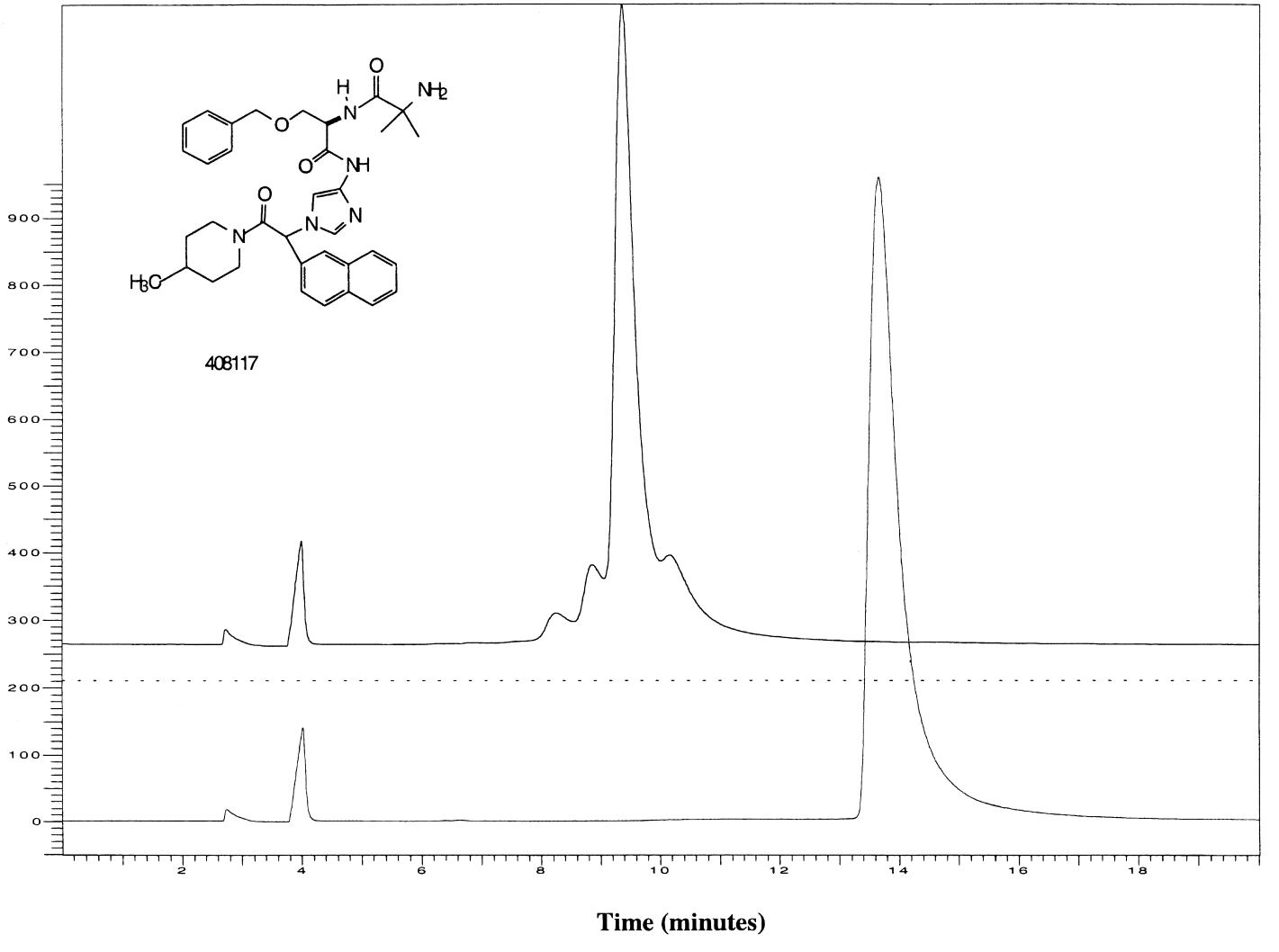
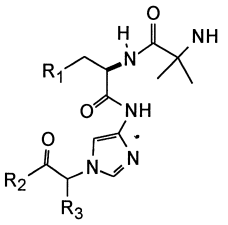
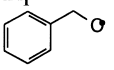
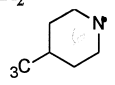
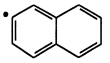
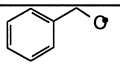
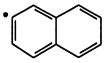
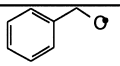
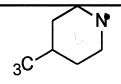
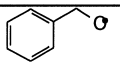
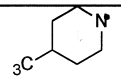
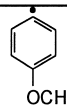
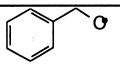
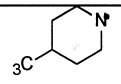
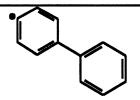
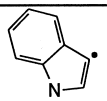
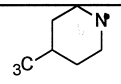
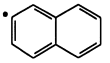
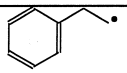
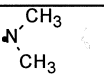
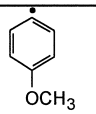
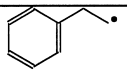
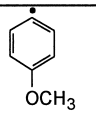
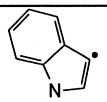
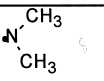
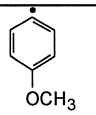
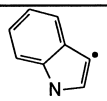
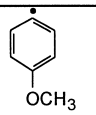


Fig. 4. Chromatogram of isolated 408117 isomers on a 25×0.46 cm Kromasil CHI-DMB column. Conditions as in the text.

Table 1
Secretagogue SAR analogs[a]



Compound	R_1	R_2	R_3	R_s	α
LY408117				2.0 **[2.5]**	1.43 **[1.53]**
LY408118				2.5	1.52
LY408787				2.0	1.34
LY408650				2.0 **[2.5]**	1.36 **[1.46]**
LY408642				2.0	1.33
LY424956				**[2.5]**	**[1.46]**
LY422194				1.5	1.18
LY422888				1.5	1.43
LY422266				1.5	1.23
LY422268				1.5	1.23

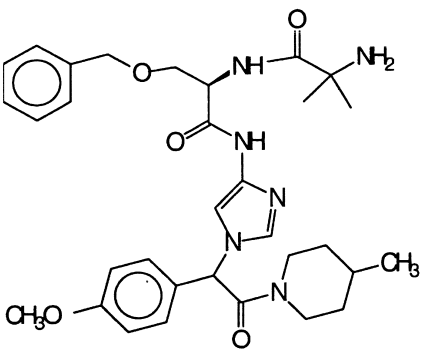
[a] All separations were performed on a 25×0.46 cm Kromasil CHI-DMB column. Conditions as in the text. Square parentheses, [], eluent was 12% ethanol, 88% heptane with 0.2% dimethylethylamine.

were conducted using a 25×0.46 cm Kromasil CHI-DMB column using the same eluent and flow-rate of 1.0 ml/min. Chromatograms of the fractions of compound LY408117 are presented in Fig. 4. While isomer 2 is quite pure, closely eluting impurities are seen with isomer 1. We reprocessed isomer 1 and increased its purity using recycle chromatography.

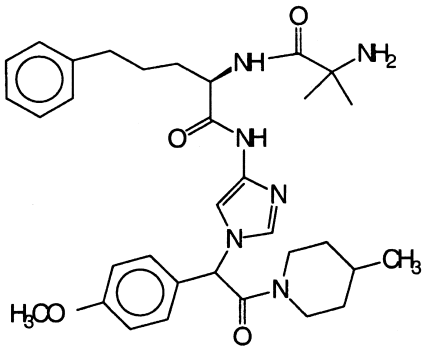
Further optimization of conditions for larger scale preparative chromatography indicated that 3A alcohol (ethanol containing 5% MeOH) could be used in place of the IPA–MeOH mixture without loss of resolution. In fact, we found that all secretagogue analogs in the SAR study could be resolved using an eluent consisting of 10% to 15% 3A alcohol in heptane containing 0.2% DMEA on a Kromasil CHI-DMB column. To increase sample loading, we slurried 500 g of Kromasil CHI-DMB in 400 ml of *n*-propanol and packed it into a 16×8 cm Prochrom column. The column was equilibrated with the appropriate eluent at a flow-rate of 250 ml/min. Column effluent was monitored at 280 nm. Samples were dissolved in the eluent at a concentration of approximately 20 mg/ml. When solubility of 20 mg/ml was obtained, column loading of 1.0 g of sample per 20 min cycle was possible. The GH secretagogue compounds that were separated using the MeOH–IPA–heptane and ethanol–heptane systems in development of the preparative procedure are shown in Table 1. The resolution factor (R_s) and selectivity (α) for separations were improved in all cases when the ethanol–heptane eluent system was used.

3.3. Preparative chromatography experiments

Optimal solubility and separation efficiency were obtained on analogs with benzyl ether or all carbon serine sub-unit and substituted aromatic in the aryl sub-unit. Two of these analogs, LY426410 and LY430148, were chosen for larger scale synthesis and toxicology studies. The structures are presented in Fig. 5. Microbiological data indicated that the second eluting isomer 2 contained most of the activity. In subsequent preparative chromatography experiments, the second isomer was purified and the shoulder peaks were not separated from isomer 1. The purifications were optimized for isolation of isomer 2. A 1-kg amount of Kromasil CHI-DMB was slurried in 900 ml of *n*-propanol and 100 ml of chloroform. This slurry was packed in a 31×8 cm Prochrom column. The chromatographic eluent consisted of 13% 3A alcohol, 87% heptane, and 0.2% DMEA. The flow-rate was 370 ml/min. and the column effluent monitored at 280 nm. Ten-g samples of the mixtures were dissolved in 500 ml of 35% 3A alcohol–heptane and 100 ml of this solution (approximately 2.0 g) was pumped on the column per cycle. Cycle time was 14 min. A 60-g amount of LY426410 with diastereomeric excess (DE) of 99% was obtained. Chromatograms of the two isolated LY426410 isomers are illustrated in Fig. 6. The analysis conditions on the Kromasil CHI-DMB were 15% ethanol in heptane containing 0.2% DMEA with a flow-rate of 1.0 ml/min and UV detection at 235 nm.



LY426410



LY430148

Fig. 5. Secretagogue analogs.

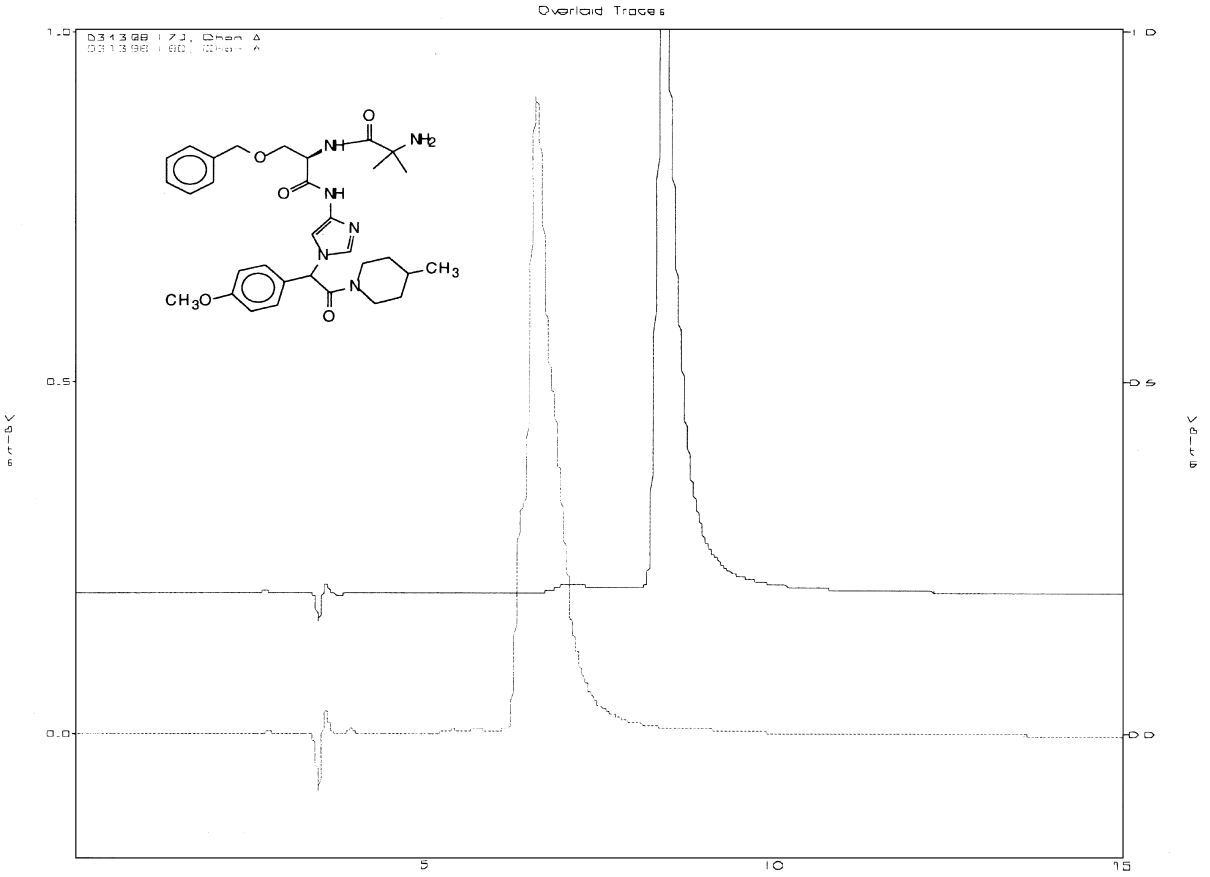


Fig. 6. Chromatograms of the isolated LY426410 diastereomers on a 25×0.46 cm Kromasil CHI-DMB column. Conditions as in the text.

Substitution of a methyl group in the R1 position (Fig. 2) in synthesis of the bottom half of the secretagogue molecule yields diastereomeric compounds prior to coupling. These types of compounds were separated on a silica gel chromatography system. Approximately 120 g of the intermediate illustrated in Fig. 7 was loaded on a 2.5 kg (30×15 cm) Biotage cartridge. The column was washed with approximately 24 liter of 15% CH_2Cl_2, 1% IPA, 0.1% DMEA in heptane. After loading the compound, the CH_2Cl_2 was increased to 25%. This system effectively separated the compound from its impurities, the desired isomer eluting after 52 liter. The undesired isomer was then eluted with 8 liter of CH_2Cl_2–IPA (1:1).

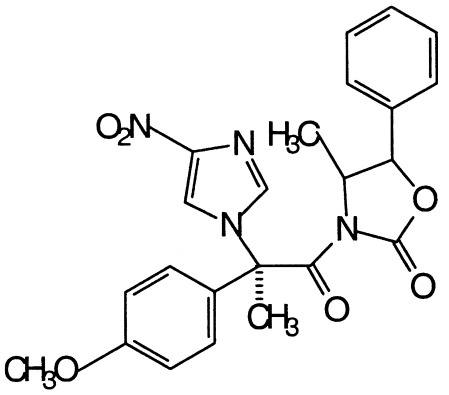


Fig. 7. Secretagogue intermediate.

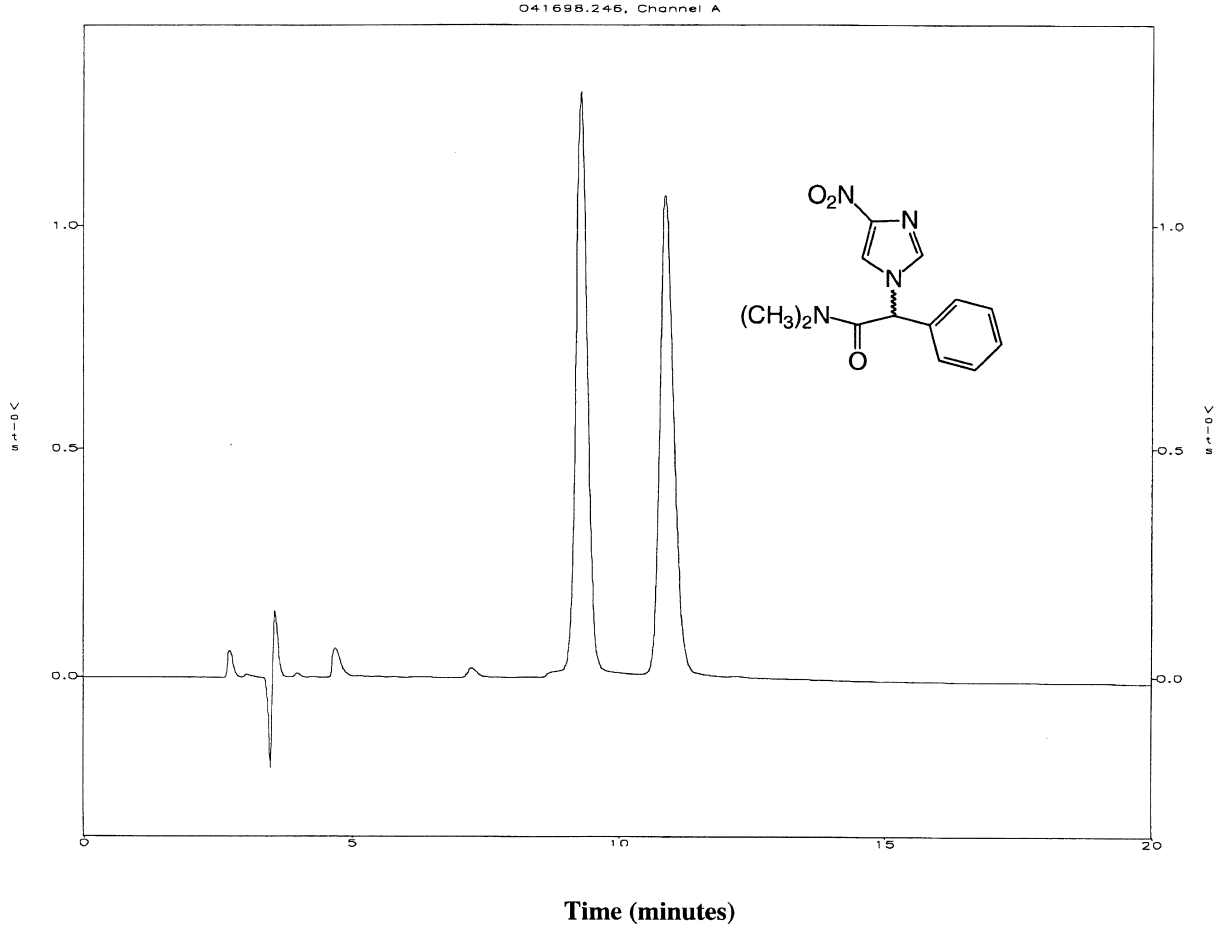


Fig. 8. Separation of GH secretagogue nitro amide intermediate on a 25×0.46 cm Kromasil CHI-DMB column. Conditions as in the text.

3.4. Analysis of other intermediates

Chiral analytical separations were developed for other different portions of the GH secretagogue molecule in support of efforts to develop an asymmetric synthesis. Modified serine portions of the secretagogue molecule included tryptophan amino acid esters and benzyl ether derivatives of serine amino acids. These compounds were resolved on Chiralcel OD-H columns using IPA–hexane eluent systems or mixed alcohol eluent systems containing ethanol–IPA in heptane with DMEA modifier. Derivatives of the nitro imidazole portion of the molecule were separated on a Kromasil CHI-DMB column (Fig. 8). The eluent system consisted of 15% ethanol in hexane with a flow-rate of 1.0 ml/min and UV detection at 235 nm. When the amide portion of these molecules was changed to an ester, IPA was substituted for ethanol to achieve the same separation.

4. Conclusion

Chromatographic separation techniques have been developed for GH secretagogue diastereomers and multigram quantities of these compounds have been isolated by preparative chromatography. Quantities ranging from 100 mg to 1.5 g of 74 diastereomeric pairs (148 chiral compounds) were purified and evaluated in the SAR study. These methods of purification reduced the time required for candidate

selection by providing pure isomeric compound much faster than by asymmetric synthetic development. Availability of these techniques made synthesis and screening of more compounds possible.

References

[1] L. Yang, G. Morriello, Y. Pan, P. Nargund, K. Barakat, K. Prendegast, K. Cheng, W.W.S. Chan, R.G. Smith, A.A. Patchett, Bioorg. Med. Chem. Lett. 8 (1998) 759–764.

[2] R.J. DeVita, R. Bochis, A.J. Frontier, A. Kotlair, M.H. Fisher, W.R. Schoen, M.J. Wyvrantt, K. Cheng, W.W.S. Chan, B. Butler, T.M. Jacks, G.J. Hickey, K.D. Schleim, K. Leung, Z. Chen, S.H. Lee Chiu, W.P. Feeney, P.K. Cunningham, R.G. Smith, J. Med. Chem. 41 (1998) 1716–1728.

[3] J.R. Tata, R.P. Nargund, M.M. Murphy, D.B.R. Johnson, A.A. Patchett, K. Cheng, L. Wei, W.W.S. Chan, B. Butler, T.M. Jacks, G. Hickey, R. Smith, Bioorg. Med. Chem. Lett. 6 (1997) 663–668.

[4] M.J. Constanzer, C.M. Chavez-Eng, B.K. Matuszewski, J. Chromatogr. B 693 (1997) 131–137.

[5] Kromasil Chiral Phases for HPLC, SFC and SMB, Application Guide, Akzo Nobel.