*Proc. Natl. Acad. Sci. USA*
Vol. 93, pp. 2031–2036, March 1996
Pharmacology

# Betidamino acids: Versatile and constrained scaffolds for drug discovery

**(chemical diversity/aminoglycine/*N*-acyl- and *N*-alkylaminoglycine/betides/gonadotropin-releasing hormone antagonists)**

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*Communicated by Leslie Orgel, The Salk Institute, San Diego, CA, October 6, 1995 (received for review December 14, 1994)*

**ABSTRACT** **Betidamino acids (a contraction of "beta" position and "amide") are N′-monoacylated (optionally, N′-monoacylated and N-mono- or N,N′-dialkylated) aminoglycine derivatives in which each N′-acyl/alkyl group may mimic naturally occurring amino acid side chains or introduce novel functionalities. Betidamino acids are most conveniently generated on solid supports used for the synthesis of peptides by selective acylation of one of the two amino functions of orthogonally protected aminoglycine(s) to generate the side chain either prior to or after the elongation of the main chain. We have used unresolved $N^{\alpha}$-*tert*-butyloxycarbonyl-$N'^{\alpha}$-fluorenylmethoxycarbonyl aminoglycine, and $N^{\alpha}$-($N^{\alpha}$-methyl)-*tert*-butyloxycarbonyl-$N'^{\alpha}$-fluorenylmethoxycarbonyl aminoglycine as the templates for the introduction of betidamino acids in Acyline [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(Ac)-D4Aph(Ac)-Leu-Ilys-Pro-DAla-$NH_2$, where 2Nal is 2-naphthylalanine, 4Cpa is 4-chlorophenylalanine, 3Pal is 3-pyridylalanine, Aph is 4-aminophenylalanine, and Ilys is $N^{\epsilon}$-isopropyllysine], a potent gonadotropin-releasing hormone antagonist, in order to test biocompatibility of these derivatives. Diastereomeric peptides could be separated in most cases by reverse-phase HPLC. Biological results indicated small differences in relative potencies (<5-fold) between the D and L nonalkylated betidamino acid-containing Acyline derivatives. Importantly, most betide diastereomers were equipotent with Acyline. In an attempt to correlate structure and observed potency, Ramachandran-type plots were calculated for a series of betidamino acids and their methylated homologs. According to these calculations, betidamino acids have access to a more limited and distinct number of conformational states (including those associated with α-helices, β-sheets, or turn structures), with deeper minima than those observed for natural amino acids.**

Current academic and pharmaceutical research has focused on the characterization of natural extracts (plants, marine organisms, or bacterial broths) and on the development of methodologies for generating chemical diversity (peptide or peptidomimetic libraries) for the discovery of new bioactive leads. The latter approach depends on automation of chemical methods for solid-phase syntheses and the identification of novel scaffolds. Several monomeric building blocks that mimic the peptide backbone have been proposed and include peptoids (1); azoles (2); 2-isoxazolines (3); oligocarbamates, oligosulfones, and oligosulfoxides (4); pyrrolinones (5); vinylogous backbones (6); β-methyl amino acids (7); and the more classical oligomers with pseudopeptide bonds described by Spatola *et al.* (8). Some of these structures may palliate the inherent undesirable features of peptides (such as lability) in the physiological milieu. Biopolymers generated from the use of these unnatural scaffolds have been found to have physicochemical, structural, biological, metabolic, and absorptive properties that differ from those of the parent peptides. Because of the limitless number of possible oligomers that can be generated from these modular elements, libraries of peptides and peptidomimetics have been used to fulfill a broad spectrum of needs ranging from the identification of epitopes for antibody binding to the generation of novel bioactive leads. Here we report synthetic pathways to orthogonally protected betidamino acid and methylbetidamino acid scaffolds (N′-monoacylated aminoglycine derivatives) (9) for solid-phase peptide synthesis and their use in the design of bioactive gonadotropin-releasing hormone (GnRH) analogs. The structural preferences of betidamino acids and their corresponding mono- and dimethylated derivatives (as compared to those of amino acids and β-methyl amino acids) were investigated by using molecular mechanics in combination with a continuum solvation model (10) to calculate the total energy of the Ac-Xaa-methyl amides as a function of backbone dihedral angle.

## MATERIALS AND METHODS

**α-(Fluorenylmethyloxycarbonylamino)-$N^{\alpha}$-(*tert*-butyloxycarbonyl)glycine [Boc-Agl(Fmoc)].** This was obtained via α-(isopropylthio)-*N*-(fluorenylmethyloxycarbonyl)glycine by the method of Qasmi *et al.* (11) and experimental protocols similar to those presented here for [Me,Boc-Agl(Fmoc)].

**α-(*N*-Methyl-*tert*-butyloxycarbonylamino)-$N^{\alpha}$-(fluorenylmethyloxycarbonyl)glycine [Me, Boc-Agl(Fmoc)].** See Fig. 1.

***tert*-Butyl-*N*-methylcarbamate.** The compound was synthesized previously by reacting methyl isocyanate with *tert*-butyl alcohol in the presence of potassium *tert*-butoxide (12) or bis(tributyltin) *tert*-butyl peroxide (13). The common disadvantages were harsh conditions, side reactions, and low yield. We reacted di-*tert*-butyl dicarbonate with methylamine to give an almost quantitative yield. To a 40% methylamine solution in water (155.5 g, 2.0 mol), a solution of di-*tert*-butyl dicarbonate (218 g, 1.0 mol) in tetrahydrofuran (THF, 300 ml) was added over 1.0 hr with stirring and cooling in an ice bath. The reaction mixture was stirred at room temperature overnight and the solvent was evaporated. The residue was dissolved in ether (1.0 liter) and washed consecutively with water, 5% sodium hydrogen sulfate, water, and saturated sodium chloride. Evaporation of the solvent and fractional distillation of the residue afforded 122.0 g (93.0%) of *tert*-butyl-*N*-methylcarbamate: b.p. 51°C/0.5 mm [literature: 41–45°C/0.1

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Abbreviations: bXaa, betidamino acid corresponding to amino acid Xaa; 2Nal, 2-naphthylalanine; 3Pal, 3-pyridylalanine; 4Cpa, 4-chlorophenylalanine; 4Aph, 4-aminophenylalanine; Ac, acetyl; Agl, aminoglycine; AOA, antiovulatory assay; 4Fpa, 4-fluorophenylalanine; GnRH, gonadotropin-releasing hormone.

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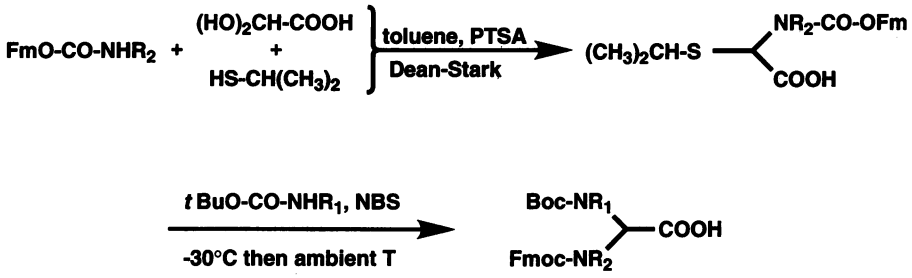


FIG. 1. Synthetic scheme for α-(fluorenylmethyloxycarbonylamino)-$N^{\alpha'}$-(*tert*-butyloxycarbonyl)glycine and corresponding methylated analogs. PTSA, *p*-toluenesulfonic acid; NBS, *N*-bromosuccinimide.

mm (12), 78–79°C/18 mm (13); mass spectrum, *m/z* $(M + H)^+$ 132.1 (mono 131.1); >98% pure by HPLC.

**α-(N-Methyl-tert-butyloxycarbonylamino)-$N^{\alpha}$-(fluorenylmethyloxycarbonyl) glycine [Me, Boc-Agl(Fmoc)].** A solution of *N*-bromosuccinimide (32.0 g, 180 mmol) in anhydrous THF (300 ml) was slowly added to a stirred solution of α-(isopropylthio)-*N*-(fluorenylmethyloxycarbonyl)glycine (44.6 g, 120 mmol) and *tert*-butyl N-methylcarbamate (31.5 g, 240 mmol) in anhydrous THF (300 ml) at −30°C over 1.0 hr and then at ambient temperature for 2 days. The THF was evaporated and the resulting syrup was dissolved in a mixture of ethyl acetate (400 ml) and water (400 ml). The pH was adjusted to 8.5 with a saturated solution of sodium carbonate. The ethyl acetate layer was separated and washed with 1.0% sodium hydrogen carbonate (2 × 100 ml). The combined aqueous phases were washed with ethyl acetate (2 × 150 ml) and acidified to pH 3.5 with saturated sodium hydrogen sulfate. Extraction with ethyl acetate, drying over sodium sulfate, filtration, and removal of the solvent afforded Me,Boc-Agl(Fmoc) as a white powder (44.0 g, 86%): m.p. 142–144°C; mass spectrum, *m/z* $(M + H)^+$ 427.2 (mono 426.2); >99% pure by HPLC.

**Synthesis and Characterization of GnRH analogs.** Analogs were synthesized by solid-phase methodology (14). All peptides were >95% pure (except in those rare cases where the two diastereomers were isolated as a 50/50% mixture shown as D/L). The antiovulatory assay (AOA) was carried out (15) in an aqueous vehicle consisting of 5% dimethyl sulfoxide in saline. Long-duration-of-action assays in the castrated male rat were carried out as described (14).

**Molecular Modeling.** Computer models of betide monomers were constructed by interactive graphics (INSIGHT II; Biosym/MSI, San Diego) and parameterized with the CFF91 force field (16). Ramachandran-type plots of the energy of *N*-acetyl-(Xaa/bXaa)-*N'*-methylamide dipeptides/dibetides as a function of backbone torsional angle were calculated with DISCOVER (Biosym), with the backbone angles systematically varied in 30° increments. Structures were minimized to a final maximum derivative of 0.01 kcal/(mol·Å). To incorporate solvation effects, the method of Schmidt and Fine (10) was employed to calculate the electrostatic component of the solvation energy.

## RESULTS AND DISCUSSION

While the synthesis of differentially substituted/protected α-aminoglycine derivatives has been reported, (17, 18), the description of the synthesis of racemic α-Fmoc-α'-Boc-aminoglycine by Qasmi *et al.* (11) led us to introduce an acyl moiety resembling an amino acid side chain on one of the two amino functions, leaving the other to form the peptide backbone. We have chosen the name "betidamino" (a contraction of "beta" position with "peptide" or "amide") acids for such monoacylated derivatives of α-aminoglycine (Fig. 2) where $R_{acyl}$ can be virtually any alkyl, aryl, or heterocyclic moiety. In our definition of a betidamino acid such as betidalanine (bAla, formylaminoglycine), the amide bond replaces a methylene group in the original amino acid (9). Here, we describe the synthesis of scaffolds where $R_1$ = H or $CH_3$ ($R_2$ = H) and their use in the design of GnRH antagonists. Preliminary results indicate that unsymmetrical scaffolds with $R_3 = CH_3$ and $R_3$ = H or $R_1 = R_3 = CH_3$ are readily available whereas similar scaffolds with $R_2 = CH_3$ and $R_1$ and/or $R_3$ = H or $CH_3$ may be more difficult to obtain, as some of the synthetic intermediates are significantly less stable (to be reported elsewhere). Of interest is that some symmetrical scaffolds, with $R_2 = CH_3$ or phenyl and $R_1 = R_3$ = H are also readily available (unpublished work).

By varying the acylating agent ($R_{acyl}$-CO), we have mimicked most of the natural amino acids (with the exception of bGlu and bGln), or in some cases their homo (Ser, Thr, Cys) and/or nor derivatives Arg. Noteworthy is the methylation needed to prepare what we defined as betide-homothreonine (bhThr), betide-isoleucine (bIle), and betide-valine (bVal),

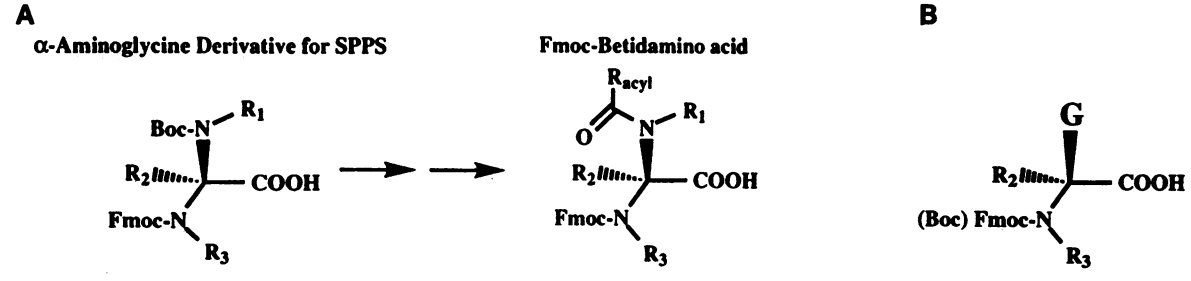


FIG. 2. (*A*) Betidamino acid scaffold. SPPS, solid-phase peptide synthesis. (*B*) Definition of the various betidamino acids. In the betide series, b stands for betide, $R_2 = R_3$ = H, NH-CO of betide amino acids is equivalent to the β-$CH_2$ in amino acids; G = -NH-CO-H, bAla; G = -$NCH_3$-CO-H, bAbu, (Val isostere); G = -$N(CH_3)$-CO-$CH_3$, bVal, (Ile isostere); G = -NH-CO-$CH(CH_3)_2$, bLeu; G = -$N(CH_3)$-CO-$CH_2$-$CH_3$, bIle; cyclo-N'-$CH_2$-CO-NH, bnorPro (Pro isostere) cyclo-N'-$(CH)_2$-CO-NH-, bPro; -NH-CO-$CH_2$-OH, bhSer; -$N(CH_3)$-CO-$CH_2$-OH, bhThr; -NH-CO-$CH_2$-SH, bhCys; -NH-CO-$CH_2$-S-$CH_3$, bMet; -NH-CO-$C_6H_5$, bPhe; -NH-CO-$C_6H_4$-OH, bTyr; -NH-CO-3-Indolyl, bTrp; -NH-CO-$(CH_2)_2$-$NH_2$, bOrn (Lys isostere); -NH-CO-$(CH_2)_3$-$NH_2$, bLys (homoLys isostere); -NH-CO-$(CH_2)$-guanidino, bnorArg (Arg isostere); -NH-CO-$(CH_2)_2$-guanidino, bArg; -NH-CO-4-imidazolyl, bHis; -NH-CO-CO-OH, bAsp (Glu isostere); -NH-CO-CO-$NH_2$, bAsn (Gln isostere); -NH-CO-$CH_2$-CO-OH, bGlu (homoGlu isostere); -NH-CO-$CH_2$-CO-$NH_2$, bGln (homoGln isostere).

which can be obtained by using a methylated scaffold. Because the betide backbone is that of an amino acid, derivatization of the side-chain nitrogen by acylation, by alkylation (as in the case of peptoids), by alkylation and acylation (as in the case of β-methyl betidamino acids), or by other reagents (such as sulfonyl chlorides, isocyanates, and thioisocyanates) does not interfere with standard coupling methodologies for chain elongation.

Structurally, we hypothesized that betidamino acids had unique properties that could be used for the identification of bioactive conformations of peptide hormones and for the design of selective analogs of peptides with multiple receptors (and receptor subtypes). Because of the replacement of a methylene (in amino acids) by an amide group (in betidamino acids), the lever arm bearing the side chain is longer in the case of betidamino acids than that in the corresponding amino acids. Systematic search procedures (molecular modeling) in an extended backbone model of Ac-Tyr-NMe and Ac-bTyr-NMe suggest that the side chains of D- and L-Tyr can share 18% of their accessible volume whereas this overlap increases to 25% for bTyr (data not shown); the possible biological consequences are presented below. In addition, we expected betides to be more hydrophilic than the corresponding peptides or β-methyl amino acid-containing peptides due to the additional amide bond introduced by the betidamino acid. Finally, we could find neither experimental nor theoretical evidence that the amide side chain was any less stable than backbone amide bonds to proteolytic enzymes, with the exception of the formyl group (unpublished work). Enzymatic resolution of several aminoglycine derivatives was unsuccessful on a preparative scale. Similarly, we could not separate the enantiomers of $N^{\alpha}$-Boc, $N'^{\alpha}$-Fmoc-aminoglycine or $N^{\alpha}$-Boc, $N'$-carbobenzoxyaminoglycine using crystallization of the quinine or brucine salts. However, the separation of most betidamino acid-containing diastereomers of GnRH (decapeptides), vasopressin (nonapeptides), somatostatin analogs (octa- to dodecapeptides), and corticotropin-releasing factor antagonists (triacontapeptides) could be achieved by reverse-phase HPLC (as shown in Table 1 for GnRH analogs). The chirality of the betidamino acids in these analogs remains to be determined, although a correlation may exist between retention times on HPLC and potency as compared to that of known homologs. We have also used an approach based on mass spectrometry and exopeptidase degradation for the identification of D-betidamino acids in a somatostatin sequence (unpublished work).

**Conformational Analysis of Betides.** The introduction of the amide functionality into aminoglycine-containing peptides (betides) is expected to perturb the conformational preferences of these molecules. As shown in Fig. 3*A*, the Ramachandran-type energy contours of the alanine dipeptide *N*-acetylalanyl-*N'*-methylamide show minima in the β, $\alpha_R$, $\alpha_L$, and $C7_{ax}$ regions. Methylation at $C^{\alpha}$ of this molecule (Fig. 3*B*) gives a much more narrow minimum in the $\alpha_R$ region, consistent with conformational restriction accompanying $C^{\alpha}$ methylation, while the minima in the β region disappear (19). Backbone N-methylation of the alanine dipeptide (Fig. 3*C*) is detrimental to right-handed helix formation while favoring backbone dihedral angles consistent with β-sheet. It should be recognized that the presence of this N-methyl group eliminates the formation of the hydrogen bond known to also stabilize β-sheets. Finally, the energy contours of the backbone-dimethylated alanine dipeptide derivative (Fig. 3*D*) show the presence of very deep wells in both the $\alpha_L$ and $\alpha_R$ regions and a wide saddle structure in the β region. In comparison, the contour plot of acetyl betidalanyl-*N'*-methylamide (Fig. 3*E*) indicates that the $\alpha_R$ minimum disappears while a "valley" with a $\phi$ value of approximately $-90°$ is observed for all values of $\psi$. This suggests that the introduction of a betidamino acid in a sequence would favor either β-sheet formation or a turn when introduced at the $i + 2$ position. $C^{\alpha}$ methylation of betidalanine could result, as expected, in a compound with a propensity to stabilize a slightly strained α-helical structure while retaining some of the features (three low-lying minima along the observed "valley" at $\psi = -90°$) of the betidalanine dipeptide (Fig. 3*F*). N-methylation of betidalanine (Fig. 3*G*) should result in a compound which strongly disfavors right-handed α-helices. The conformational space available to the backbone-dimethylated betidalanine (Fig. 3*H*) is somewhat more constricted than that of the corresponding alanine derivative, while the positions of the minima are only slightly perturbed.

Because of the availability of facile methods for the preparation of side-chain-methylated betidamino acids, conformational energy calculations were conducted on valine and betidevaline concomitant with backbone $N^{\alpha}$ and $C^{\alpha}$ methylation. As shown in Fig. 4*A*, the $\phi$–$\psi$ contour map of acetyl

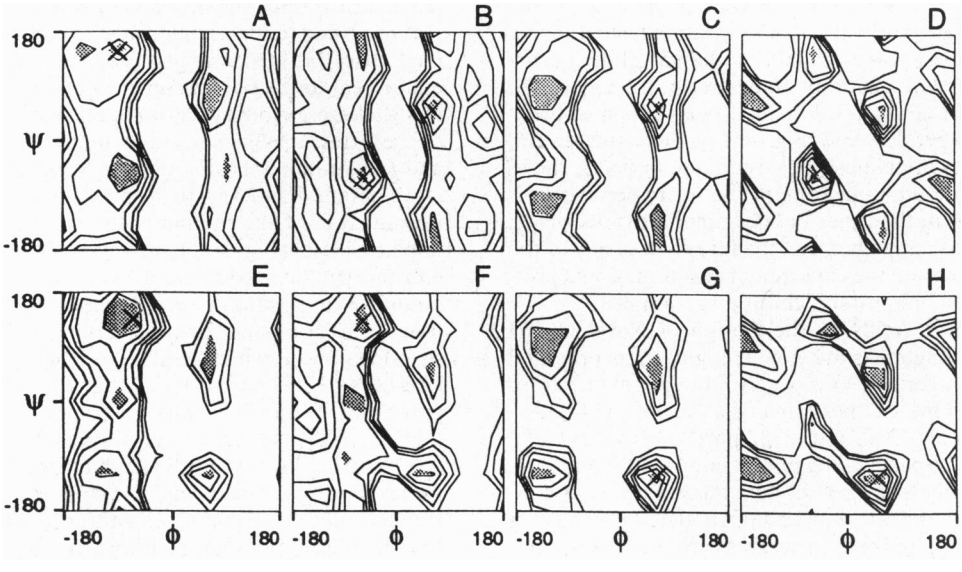


FIG. 3. Ramachandran-type $\phi$–$\psi$ composite energy (internal plus solvation) plots of *N*-acetylalanyl-*N'*-methylamides (A–D) and *N*-acetylbetidalanyl-*N'*-methylamides (*E–H*). (*A* and *E*) Nonmethylated. (*B* and *F*) $C^{\alpha}$-methylated. (*C* and *G*) N'-methylated. (*D* and *H*) $C^{\alpha}$,N'-dimethylated. Energy contours are shown at 1.0-kcal/mol intervals starting 0.5 kcal/mol above each global minimum. Contours greater than 6.5 kcal/mol above a global minimum are not shown. Local minima are stippled and the global minima are marked with crosses.

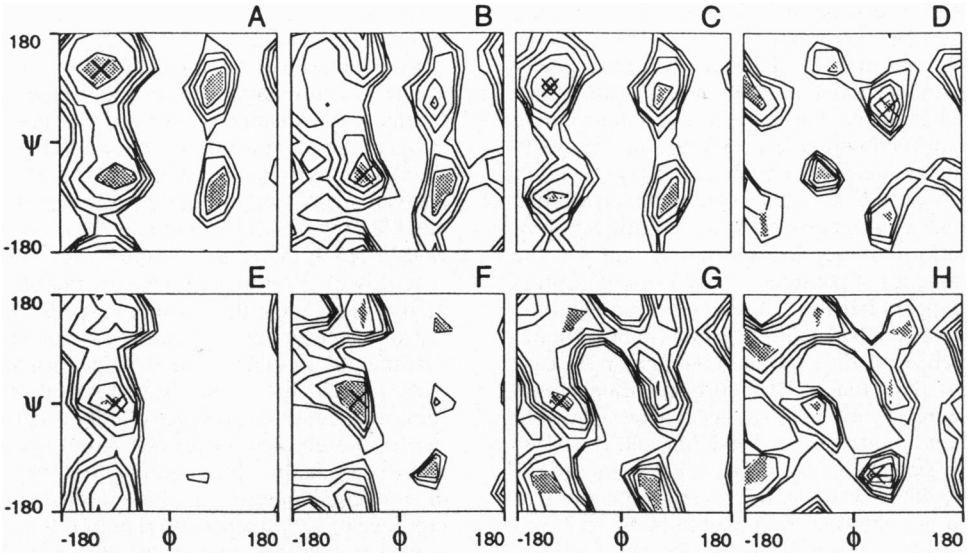


FIG. 4. Ramachandran-type $\phi$–$\psi$ composite energy (internal plus solvation) plots of *N*-acetylvalyl-*N'*-methylamides (*A–D*) and N-acetylbetidevalyl-*N'*-methylamides (*E–H*). (*A* and *E*) Nonmethylated. (*B* and *F*) $C^{\alpha}$-methylated. (*C* and *G*) N'-methylated. (*D* and *H*) $C^{\alpha}$,N'-dimethylated. Energy contours are shown at 1.0-kcal/mol intervals starting 0.5 kcal/mol above each global minimum. Contours greater than 6.5 kcal/mol above a global minimum are not shown. Local minima are stippled and the global minima are marked with crosses.

valyl-*N'*-methylamide is very similar to the corresponding alanine map (Fig. 3*A*), with minima in the $\alpha_R$, $\alpha_L$, C5, and $C7_{ax}$ regions, but with slightly steeper energy barriers. This restriction on conformational freedom is even more evident in the $C^{\alpha}$-methylvaline derivative (Fig. 4*B*), which shows two approximately symmetrical minima in the $\alpha_L$ and $\alpha_R$ regions with much steeper potential energy barriers about them than what is seen for the corresponding alanine derivative. The similarity between the alanine and valine dipeptides continues in the case of backbone N-methylation, where for valine (Fig. 4*C*) the positions of the four minima are unchanged with respect to alanine (Fig. 3*C*) but have much steeper wells. The backbone-dimethylated valine derivative (Fig. 4*D*) should be highly conducive to $\alpha$-helical formation. Examination of Fig. 4*E* suggests that betidevaline could induce turns in the $i + 2$ position and should strongly disfavor both $\alpha$-helical and $\beta$-sheet secondary structures. $C^{\alpha}$ methylation of betidevaline (Fig. 4*F*) has an effect similar to that of $C^{\alpha}$ methylation of betidalanine (Fig. 3*F*), with the expected deepening of the energy wells. It is noteworthy that N-methylation restores some of the conformational space available to betidevaline, particularly in the upper left quadrant (meandering valley) of the Ramachandran plot (Fig. 4*G*). Finally, the $\phi$–$\psi$ map of backbone-dimethylated betidevaline (Fig. 4*H*), while having several minima in unconventional positions, is unique in that it shows a well-defined minimum appearing in the $\alpha_R$ region which is not as well defined in the other betidevaline derivatives (Fig. 4 *E–G*). One should remember that the inversion of any of the Ramachandran plots and the consequent conformational preferences predicted should attend chiral inversion at $C^{\alpha}$.

**Use of Betidamino Acids in the Design of Novel GnRH Analogs.** GnRH antagonists are now recognized as potential drugs for the management of sex steroid-dependent pathophysiologies (20). One of these analogs, Acyline (14), [Ac-D2Na$^1$,D4Cpa$^2$,D3Pal$^3$,4Aph$^5$(Ac),D4Aph$^6$(Ac),ILys$^8$,DAla$^{10}$]GnRH, (**1**, Table 1) is particularly potent, long acting, and safe. Since Acyline readily form gels in aqueous solutions at concentrations of $\geq$10 mg/ml, it was hoped that by replacing individual amino acids in these structures with the corresponding betidamino and monomethylbetidamino acids, we could generate betides with increased hydrophilicity (deduced from retention times on reverse-phase HPLC using a neutral buffer) that would lack the propensity to gel.

Structures of compounds **4–16** (Table 1) are those of some selected betide analogs of Acyline. It should be recognized that betide-2-napthylalanine (b2Nal) is also Agl(2-naphthoyl), betide-3-pyridylalanine (b3Pal) is Agl(nicotinoyl), and betidalanine (bAla) is Agl(Formyl) and that the methyl group (Me), when present, is located on the same nitrogen as the acyl group mimicking the side chain of the corresponding amino acids. Compounds **2** and **3** were synthesized for comparison purposes to further define the chiral requirements at positions 1 and 10, respectively. Clearly, introduction of an L residue at position 1 leads to a 10-fold decrease in potency (compare **2** and **1**), whereas chiral inversion has minimal effect at position 10 (compare **3** with **1**), as expected from earlier studies (21) and further documented here (peptides **12** and **13** with D- or L-bAla$^{10}$). Remarkably, the difference in potency upon incorporation of either D- or L-b2Nal at position 1 (diastereomers **4** and **5**) is only 2-fold or less, possibly due to the increased conformational space accessible to the side chains of betidamino acids. Additionally, potency is relatively insensitive to the nature of residue 10; racemic mixtures of [bMeAla$^{10}$]Acyline (**14**), [Agl$^{10}$]Acyline (**15**), and [Agl(Me)$^{10}$]Acyline (**16**) are virtually equipotent to the parent Acyline (**1**). Of further interest is the fact that the introduction of a methyl group on the side chain of the betide Acylines restores discrimination between the **D**- and **L**-containing diastereomers (**6** and **11** are at least 4 times more potent than **7** and **10**, respectively). This suggests that the introduction of the methyl group on the side-chain nitrogen impairs side-chain mobility in ways still to be determined. Also, a primary amine (compound **14**) or a secondary amine (compound **15**) at position 10 is compatible with unexpectedly high potency as compared to the introduction of a closely related D-serine residue, which results in a 10-fold loss of potency when [Ac-D2Nal$^1$,D4Fpa$^2$,DTrp$^3$,DArg$^6$,DSer$^{10}$]GnRH (partially potent at 10 $\mu$g per rat) is compared with [AcD2Nal$^1$,-D4Fpa$^2$,DTrp$^3$,DArg$^6$,DAla$^{10}$]GnRH and [AcD2Nal$^1$,-D4Fpa$^2$,DTrp$^3$,DArg$^6$]GnRH [which are fully active at 1 $\mu$g (0 rats ovulating out of 8 and 10 respectively, data not shown]. Finally, betide Acyline diastereomers **8** and **9** (D- or L-b3Pal$^3$) have potencies within a factor of 2. Although early investigations suggested that the D isomer at position 3 led to loss of intrinsic activity of the given antagonists and was therefore a favorable substitution, (21), this was not confirmed in the most potent and recent GnRH antagonists. The observation

Table 1. Acyline [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(Ac)-D4Aph(Ac)-Leu-Ilys-Pro-DAla-$NH_2$] and selected betide Acylines

| No. | Compound | AOA* | | RT† | Duration of action‡ |
|---|---|---|---|---|---|
| **1** | Acyline | 2.5 | (0/7) | 24.1 | Long |
| | | 1.0 | (5/8) | | |
| **2** | [Ac-L2Nal[1]]Acyline | 25 | (0/3) | 26.5 | |
| | | 10 | (2/6) | | |
| **3** | [L-Ala[10]]Acyline | 2.5 | (0/8) | 23.3 | Intermediate |
| | | 1.0 | (8/8) | | |
| **4** | [Ac-D or Lb2Nal[1]]Acyline | 2.5 | (0/8) | 21.4 | Intermediate |
| | | 1.0 | (2/7) | | |
| **5** | [Ac-L or Db2Nal[1]]Acyline | 5.0 | (0/8) | 22.7 | |
| | | 2.5 | (5/5) | | |
| **6** | [Ac-D or LbMe2Nal[1]]Acyline | 2.5 | (0/8) | 22.3 | |
| | | 1.0 | (2/8) | | |
| **7** | [Ac-L or DbMe2Nal[1]]Acyline | 10 | (7/8) | 24.1 | |
| **8** | [D or Lb3Pal[3]]Acyline | 2.5 | (0/7) | 20.8 | |
| | | 1.0 | (3/3) | | |
| **9** | [L or Db3Pal[3]]Acyline | 2.5 | (0/5) | 23.5 | Intermediate |
| | | 1.0 | (6/14) | | |
| **10** | [D or LbMe3Pal[3]]Acyline | 10 | (8/8) | 21.5 | |
| **11** | [L or DbMe3Pal[3]]Acyline | 2.5 | (3/8) | 24.7 | |
| **12** | [D or LbAla[10]]Acyline | 1.0 | (6/17) | 22.2 | Long |
| | | 0.5 | (9/11) | | |
| **13** | [L or DbAla[10]]Acyline | 2.5 | (0/8) | 22.4 | |
| | | 1.0 | (3/3) | | |
| **14** | [D/L-bMeAla[10]]Acyline | 2.5 | (0/8) | 23.3 | |
| | | 1.0 | (1/8) | | |
| **15** | [D/L-Agl[10]]Acyline | 2.5 | (0/8) | 22.4 | |
| | | 1.0 | (3.8) | | |
| **16** | [D/L-Agl(Me)[10]]Acyline | 2.5 | (0/8) | 23.3 | |
| | | 1.0 | (8/8) | | |

*Antiovulatory assay: dosage in micrograms (rats ovulating/total).
†RT, retention times (min) under gradient conditions (40% to 75% in 30 min); buffer A, triethylammonium phosphate (pH 7.30); buffer B: 60% $CH_3CN$/40% A. The "D or L" assignment was given to the more hydrophilic of the two diastereomers. "D/L" indicates a 50/50 mix of the two diastereomers as determined by capillary zone electrophoresis.
‡Measurement of circulating luteinizing hormone in castrated rats treated subcutaneously with the peptides (50 μg) was carried out over a period of 72 hr or more as reported earlier (14). Long duration of action = fully active after 72 hr; intermediate = fully active at 48 hr but only marginally so at 65 hr.

that **8** and **9** are equipotent may therefore be of great significance, as it suggests that the introduction of an L amino acid at position 3 may be compatible with high potency.

To address concerns of possible instability of Agl-containing peptides (*gem*-diamines are known to be chemically unstable), analogs **15** and **16** (with a primary and secondary amino function, respectively) were synthesized. We were surprised to find out not only that they were stable but also that they were unexpectedly equipotent with Acyline, since the introduction of a D-serine or D-phenylglycine at position 10 of [AcD2Nal[1],D4Fpa[2],DTrp[3],-DArg[6],DXaa[10]]GnRH resulted in a 4-fold and 50-fold loss of potency, respectively, as compared to [AcD2Nal[1],D4Fpa[2],-DTrp[3],DArg[6],DAla[10]]GnRH (unpublished results).

All betide Acylines that are either equipotent or more potent than Acyline have a shorter chromatographic retention time than Acyline, suggesting that the hydrophilicity of GnRH antagonists can be modulated by their conversion into betides with retention of potency in short-term *in vivo* assays. In long-term *in vivo* assays it is apparent that in some cases tested, introduction of a betide amino acid results in shortened duration of action. More specifically, while Acyline shows full inhibition at, and beyond, the 72-hr time point but not at the 120-hr time point, secretion of luteinizing hormone is restored, at least in part, for all of the other analogs tested in Table 1 at around 70 hr, with the exception of compound **12**, which contains a betide residue at position 10. It is that much more significant that introduction of an L-alanine at that position (compound **3**) results in a significant loss of duration of action. These limited studies suggest that while hydrophilicity, as measured by retention times on reverse-phase supports, is increased by the introduction of betidamino acids, more data will be needed to fully understand the potential and limitations of betidamino acids in biologically active peptides.

Scanning a GnRH antagonist using the corresponding betidamino acids led to the following observations: (*i*) betidamino acids, and, to a lesser extent *N*-methylbetidamino acid-containing peptides, are more hydrophilic than their parent compounds and (*ii*) whereas D and L amino acid-containing peptides have biological potencies that often differ by at least one or more orders of magnitude, D and L betidamino acid-containing peptides (generally) differ in potency by only a factor of <2 to 5, suggesting that D and L betidamino acid-containing peptides may assume similar conformations (as shown above, 25% overlap for the betide side chain of bTyr versus 18% in the case of Tyr) which are the same as that of the bioactive conformer(s). This is an important observation, as there may be no need for the separation of the diastereoisomers before preliminary testing or screening of betides. Introduction of a methyl group on the side-chain nitrogen, however, may reinstate the chiral singularity of the D and L betidamino acids with respect to biological activity. In most peptide/protein-related fields, because of the unlimited diversity of functional and structural modifications that can be introduced or induced respectively in the oligomers, we have found the possibility of incremental constraints on the backbone and side-chain dihedral angles by the facile introduction of one or two methyl groups in a betide scaffold to be extremely promising. A preliminary methylbetide scan of a somatostatin analog has already revealed receptor-specific leads (22).

We thank Drs. C. Rivier and J. Reel for the antiovulatory and castrated male rat assays. Dr. Reel's data were obtained at Bioqual Inc. under contract NO1-HD-1-3130 with the Contraceptive Development Branch, Center for Population Research, National Institute of Child Health and Development. We thank Dr. R. Fine for sharing the content of his manuscript with us prior to publication and Dr. J. Hempel for valuable theoretical discussions. We acknowledge the outstanding technical contributions of Dr. T. Jones, R. Kaiser, D. Pantoja, S. Johnson, and C. Miller; the constructive comments of D. Kirby and L. Cervini; and the assistance of D. Johns in manuscript preparation. This work was supported by the National Institutes of Health under Contracts NO1-HD-0-2906 and NO1-HD-3-3171 and Grants HD13527 and DK26741 and the Hearst Foundation.

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