Daniel M. G. Barron, Department of Biological Chemistry, Harvard Medical School, Boston, Mass., for measuring CD spectra of the nucleosides reported herein.

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Phenylalanyl Transfer Ribonucleic Acid Synthetase from *Escherichia coli* B. Potent Inhibition by Analogues of *N*-Benzyl-2-phenylethylamine

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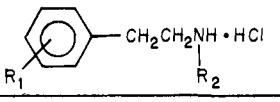
A potent new class of inhibitors of phenylalanyl-tRNA synthetase from *Escherichia coli* B is described. *N*-Benzyl-2-phenylethylamine is a competitive inhibitor with respect to L-phenylalanine and appears to possess the structural features required for near-optimal binding. Hydrophobic substituents at the ortho position of either ring appear to be well tolerated, but substituents on both rings lead to large losses in binding. Poor noncompetitive inhibitors result from alkylation of the secondary nitrogen, further separation of the *N*-benzyl group from the nitrogen, or alkylation at the α position of the *N*-benzyl moiety. In contrast, placement of a methyl group at the 1 position of the 2-phenylethylamine moiety to give *N*-benzyl-D-amphetamine results in the most potent inhibitor yet described for this enzyme.

Each of the aminoacyl-tRNA (aa-tRNA) synthetases is responsible for the attachment of an amino acid to the tRNA's which recognize the codons for that amino acid. Should an uncorrected mistake occur at this stage, the

Table I. Physical Constants[a] of 

No.	R_1	R_2	Recrystn solvent	Mp, °C	Formula
14	4-OH	$-CH_2(4\text{-}HOC_6H_4)$	10 mM HCl (aq)	229.5	$C_{15}H_{18}NO_2Cl$
15	H	$-CH_2(4\text{-}HOC_6H_4)$	EtAc-petr ether	137-138.5[b]	$C_{15}H_{17}NO$
16	2-CH_3	$-CH_2C_6H_5$	*i*-PrOH	217	$C_{16}H_{20}NCl$
17	2-CH_3	$-CH_2(2\text{-}CH_3C_6H_4)$	*i*-PrOH	238-239	$C_{17}H_{22}NCl$
18	H	$-CH_2(2\text{-}CH_3C_6H_4)$	EtOH-Et_2O	243.5-244.5	$C_{16}H_{20}NCl$
19	H	$-CH_2(3\text{-}CH_3C_6H_4)$	EtOH-Et_2O	249.5-250.5	$C_{16}H_{20}NCl$
22	H	$-CH_2(2\text{-}NO_2C_6H_4)$	*i*-PrOH-Et_2O	181.5-183	$C_{15}H_{17}N_2O_2Cl$
30	H	$-CH_2(CH_2)_2C_6H_5$	EtOH-Et_2O	261-262	$C_{16}H_{20}NCl$
32	H	$-CH_2(C_6H_5)CH_3$	*i*-PrOH-Et_2O	232-233	$C_{16}H_{20}NCl$
33	H	$-CH(C_6H_5)CH_2CH_3$	EtOH-Et_2O	223-224.5	$C_{17}H_{22}NCl$
34	H	$-CH(C_6H_5)CH_2CH_2CH_3$	EtOH-Et_2O	230.5-231.5	$C_{18}H_{24}NCl$
35	H	$-CH(C_6H_5)CH(CH_3)_2$	EtOH-Et_2O	255-255.5	$C_{18}H_{24}NCl$

[a] Melting points were obtained on a Mel-Temp block and are corrected. Crude yields were between 80 and 100% for all compounds. [b] Prepared as free base.

amino acid would be incorporated into an incorrect position of the protein (for a review, see ref 1). The overall reaction catalyzed by these enzymes appears to involve (1) activation of a particular amino acid to form an aminoacyl adenylate (aa-AMP) intermediate and (2) transfer of the activated amino acid to its cognate tRNA.

$$aa + ATP \rightleftharpoons aa\text{-}AMP + PP_i \quad (1)$$

$$aa\text{-}AMP + tRNA \rightleftharpoons aa\text{-}tRNA + AMP \quad (2)$$

In attempts to understand the molecular basis for the remarkable fidelity of the aa-tRNA synthetases, competitive inhibitors have been utilized to map the active sites of a number of these enzymes.[2-7] Investigations of this type have provided the fundamental information required for the design of potent and specific inhibitors of these enzymes.

In the present work, analogues of *N*-benzyl-2-phenylethylamine have been utilized to further define the previously described[2] topography of the amino acid binding site of phenylalanyl-tRNA synthetase (PRS) from *Escherichia coli*. Several of these analogues represent the most potent inhibitors of this enzyme yet reported. In an accompanying report, it is further shown that many of these analogues demonstrate a remarkable species selectivity in their ability to inhibit the phenylalanyl-tRNA synthetases from *E. coli* and rat liver sources.

Experimental Section

Phenylalanyl-tRNA synthetase was isolated from *E. coli* B (General Biochemicals) by the method of Stulberg.[8] The preparation was 68% pure with respect to published values of the maximal rate of ATP-[^{32}P]PP$_i$ exchange.[9] Phenylalanine analogues were evaluated for their ability to inhibit ATP-[^{32}P]PP$_i$ exchange by the procedure previously described.[9] Inhibition constants were obtained from double reciprocal plots[10] varying L-phenylalanine at fixed levels of ATP (4.0 mM). All inhibitors were competitive with respect to L-phenylalanine unless otherwise stated.

ATP, L-phenylalanine, and D-3-phenyl-2-aminopropane (D-amphetamine, **5**) were products of Sigma Chemical Co. 2-Phenylethylamine (**1**), 1,2-diphenylethylamine (**23**), 1,1-dimethyl-2-phenylethylamine (**6**, purified as their HCl salts), and L-3-phenyl-2-aminopropane (L-amphetamine, **4**) were obtained from Aldrich Chemical Co. D-Phenylalanine was obtained from Mann Research Laboratories. *N*-Methyl-*N*-benzyl-D-amphetamine (**40**) was obtained courtesy of Dr. Malcolm Rowland.

D- and L-phenylalaninol (**3** and **2**),[11] 2-*o*-methylphenylethylamine hydrochloride,[12,13] *N*-methyl-*N*-benzyl-2-phenylethylamine hydrochloride (**38**),[14] *N*,*N*-dibenzyl-2-phenylethylamine hydrochloride (**41**),[14,15] and *N*-methyl-*N*-benzyl-L-amphetamine hydrochloride (**39**)[16,17] were prepared by literature procedures and were pure by criteria of melting point and homogeneity on TLC using silica gel plates and ethyl acetate solvent. The 1-substituted 2-phenylethylamine derivatives, 1,3-diphenylisopropylamine hydrochloride (**41**)[14,15] and 1,4-diphenyl-1-butylamine hydrochloride (**25**),[18] were prepared by the method of Pohland and Sullivan[19] except sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al, Aldrich) was used in place of lithium aluminum hydride for reduction of the intermediate Grignard-nitrile adducts.

The following compounds were prepared by a general reductive alkylation procedure as described below for preparation of *N*-benzyl-2-phenylethylamine hydrochloride (**7**): *N*-benzyl-1,2-diphenylethylamine hydrochloride (**26**),[20] *N*-benzyl-1,3-diphenylethylamine hydrochloride (**27**),[21] *N*-benzyl-1,4-diphenyl-2-butylamine (**28**),[21] *N*-(*p*-xylyl)-2-phenylethylamine hydrochloride (**20**),[22] *N*-benzyl-D- and -L-amphetamine hydrochloride (**11** and **10**),[23] *N*-benzyl-D- and -L-phenylalaninol (**9** and **8**),[24] *N*-(*o*-methoxybenzyl)-2-phenylethylamine hydrochloride (**21**),[25] *N*-benzyl-2-(2-methylphenyl)ethylamine hydrochloride (**16**), *N*-(2-xylyl)-2-(2-methylphenyl)ethylamine hydrochloride (**17**), *N*-(2-xylyl)-2-phenylethylamine hydrochloride (**18**), *N*-(3-xylyl)-2-phenylethylamine hydrochloride (**19**), *N*-(2-nitrobenzyl)-2-phenylethylamine hydrochloride (**22**), and *N*-benzyl-1,1-dimethyl-2-phenylethylamine hydrochloride (**12**).[26] The purity of all compounds was established by criteria of melting point, TLC on silica gel with ethyl acetate solvent, and also elemental analysis for all previously uncharacterized compounds (Table I).

***N*-Benzyl-2-phenylethylamine Hydrochloride (7).** To a stirred solution of 2-phenylethylamine (**1**, 125 μl, 1.0 mmol) in absolute ethanol (1.0 ml) at 25° was added 110 μl (1.1 mmol) of freshly distilled benzaldehyde. After 2 h the solution was chilled in an ice bath and 2.0 ml (1.0 M) of freshly prepared $NaBH_4$ in ethanol was added. After 5 min, excess $NaBH_4$ was destroyed by addition of ethanolic HCl (5 ml, 2 N) and solvents were removed by spin evaporation. The residue was partitioned between 0.1 N NaOH (10 ml) and ether (2 × 10 ml). The pooled ether fractions were extracted (3 × 15 ml) with 0.1 M aqueous sulfuric acid. The pooled acidic fractions were made basic with 50% NaOH and extracted with ether (3 × 20 ml). The combined ether extracts were washed with water and dried over anhydrous sodium sulfate. After removal of the drying agent, HCl gas was bubbled into the ether to precipitate the crude amine hydrochloride salt. Recrystallization to a constant melting point from ethanol-ether mixtures gave 200 mg (83%) of *N*-benzyl-2-phenylethylamine hydrochloride (**7**), mp 269-270° (lit.[22] mp 265-266°).

The preparation of the phenolic analogues *N*-benzyltyramine (**13**),[27] *N*-(*p*-hydroxybenzyl)-2-phenylethylamine (**15**), and *N*-(*p*-hydroxybenzyl)tyramine (**14**) required slight modification from the procedure described above. After reduction of the imine intermediate, destruction of excess borohydride, and removal of solvents (as above), the crude mixture was suspended in 5% $NaHCO_3$. Filtration yielded the crude products which were recrystallized to constant melting point. *N*-(*p*-Hydroxybenzyl)tyramine (**14**) required also the presence of triethylamine hydrochloride (1 equiv) to form the imine intermediate.

The following derivatives of 2-phenylethylamine were prepared by a modified reductive alkylation procedure as described below

Table II. Inhibition Constants and Negative Free Energies of Binding for Phenylethylamine Derivatives

		Primary amine			N-Benzyl derivative	
	Compound	K_i, μM	$-\Delta F$,[a] kcal/mol		K_i, μM	$-\Delta F$, kcal/mol
1	2-Phenylethylamine	93	5.72	7	0.67	8.76
2	L-Phenylalaninol	6	7.42	8	38	6.27
3	D-Phenylalaninol	1420	4.04	9	0.77	8.68
4	L-Amphetamine	10	7.09	10	5.6	7.45
5	D-Amphetamine	190	5.28	11	0.14	9.75
6	α,α-Dimethyl-2-phenylethylamine	480	4.71	12	230	5.16

[a] Negative free energies of binding are calculated using the formula: $-\Delta F = RT \ln K$

for N-(2-phenylethyl)-1-phenylethylamine hydrochloride (32): N-(2-phenylethyl)-1-phenylpropylamine hydrochloride (33), N-(2-phenylethyl)-1-phenylbutylamine hydrochloride (34), N-(2-phenylethyl)-1-phenylisobutylamine hydrochloride (35), N-(2-phenylethyl)benzhydrylamine hydrochloride (36),[28] N-(2-phenylethyl)-1,2-diphenylethylamine (37),[28] N,N-di-2-phenylethylamine hydrochloride (29),[28] N-(2-phenylethyl)-3-phenylpropylamine hydrochloride (30), and N-(2-phenylethyl)-4-phenylbutylamine hydrochloride (31).[28]

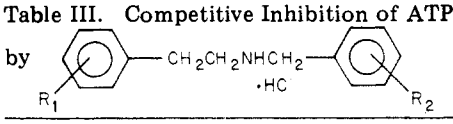
N-(2-Phenylethyl)-1-phenylethylamine Hydrochloride (32). Freshly distilled 2-phenylethylamine (1, 1.21 ml, 10 mmol) and acetophenone (1.32 g, 11 mmol) were dissolved in benzene (25 ml) in a round-bottomed flask fitted with a modified Dean-Stark trap. After addition of acetic acid catalyst (50 μl, 0.83 mmol), the solution was refluxed 8 h to remove water. Distillation of the solvents yielded the crude ketimine residue which was dissolved in absolute ethanol (15 ml) with stirring and 10 ml of a freshly prepared ethanolic $NaBH_4$ solution (1 M) was added. After 10 min the reaction was complete and excess $NaBH_4$ was destroyed by addition of ethanolic HCl (2 N, 20 ml). Solvent and excess acid were removed in vacuo to yield the crude product which was partitioned between aqueous HCl (20 ml, 1 M) and ether (3 × 20 ml) until no further uv-absorbing materials were extracted into the ether phase. The aqueous phase was made basic by addition of 50% NaOH and the amine product was extracted with ether (3 × 20 ml). The pooled ether extracts were dried over anhydrous sodium sulfate, filtered, and treated with HCl gas to precipitate the crude amine salt, 2.20 g (82%). A portion of the crude product was recrystallized from 2-propanol–ether mixtures to give 2-phenylethyl-1-phenylethylamine hydrochloride (32): mp 232–233°. Anal. ($C_{16}H_{20}NCl$) C, H, N.

Results

Inhibitory properties of a series of 2-phenylethylamine and N-benzyl-2-phenylethylamine derivatives in the PRS-catalyzed ATP–PP_i exchange are given in Table II. All inhibitors are competitive with respect to L-phenylalanine. As previously reported,[2] addition of a methyl or hydroxymethyl group to the α position of 2-phenylethylamine (1) to give L-amphetamine (4) and L-phenylalaninol (2) results in an enhanced affinity of the analogue for PRS by 1.37 and 1.70 kcal/mol, respectively, whereas D-amphetamine (5) and D-phenylalaninol (3) show a *decrease* in affinity of 0.44 and 1.68 kcal/mol, respectively. Placement of two α-methyl groups on 2-phenylethylamine (1) gives 1,1-dimethyl-2-phenylethylamine (6) which binds ca. 1 kcal/mol poorer than 2-phenylethylamine (1). These results reflect the stereospecificity of binding by the enzyme and, since the L isomers bind better than the substrate L-phenylalanine, suggest that the carboxylate binding region of *E. coli* PRS is hydrophobic.[2]

Placement of an N-benzyl group on 2-phenylethylamine (1) to give N-benzyl-2-phenylethylamine (7) results in an increased affinity for PRS of 3.04 kcal/mol. Interestingly, the same substitution on the substrate results in a large loss in binding: N-Bzl-Phe binds at least 200-fold poorer than Phe.[2] When the N-benzyl group is placed on L-phenylalaninol (2), L-amphetamine (4), or 1,1-dimethyl-2-phenylethylamine (6), the resultant compounds (3, 10, and 12) bind approximately the same as the parent amines. However, when compared to N-benzyl-2-phenylethylamine (7), the N-benzyl derivatives of L-phenylalaninol (8) and L-amphetamine (10) bind poorer by 2.5 and 1.3 kcal/mol, respectively. In contrast, N-benzyl-D-phenylalaninol (9) and N-benzyl-D-amphetamine (11) bind significantly better than the parent D-phenylalaninol (3) and D-amphetamine (5) (more than 4 kcal/mol). Compared to N-benzyl-2-phenylethylamine (7) there is no loss in binding with the D-CH_2OH substituent and a gain in binding energy of ~1 kcal/mol for the D-CH_3 substituent. N-Benzyl-D-amphetamine (11) has $K_i = 1.4 \times 10^{-7}$ M and is the most potent inhibitor yet reported for this enzyme.

Table III. Competitive Inhibition of ATP-PP_i Exchange by



	R_1	R_2	K_i, μM	$-\Delta F$, kcal/mol
13	4-OH	H	109	5.62
14	4-OH	4-OH	700	4.48
15	H	4-OH	79	5.82
16	2-CH_3	H	0.51	8.93
17	2-CH_3	2-CH_3	3.81	7.69
18	H	2-CH_3	0.63	8.80
19	H	3-CH_3	10.4	7.07
20	H	4-CH_3	13.3	6.92
21	H	2-OCH_3	0.92	8.56
22	H	2-NO_2	15.2	6.84

Listed in Table III are inhibition constants for a number of N-benzyl-2-phenylethylamine (7) derivatives which have substituents on one or both of the phenyl rings. Of the derivatives examined, substitution at the ortho position of the N-benzyl moiety appears to be the best tolerated. Another generalization that appears valid is that placement of substituents on both phenyl rings leads to larger losses in binding than single substitutions on either ring. Tyramine is a poor noncompetitive inhibitor of PRS; addition of an N-benzyl group to give N-benzyltyramine (13) results in a reasonably good competitive inhibitor albeit much poorer (~200-fold) than N-benzyl-2-phenylethylamine (7). Placement of a p-hydroxyl group on the N-benzyl moiety of N-benzyl-2-phenylethylamine (7) to give N-(p-hydroxybenzyl)-2-phenylethylamine (15) also results in a large loss in binding (~100-fold) compared with the parent compound. When hydroxyl groups are placed at the para position of both rings to give N-(p-hydroxybenzyl)tyramine (14), the loss in binding is significantly larger, but not additive, to that obtained with the monosubstituted compounds (13 and 15).

Increasing the length of the carbon chain separating the N-benzyl group from the 2-phenylethylamine moiety results in a decreased affinity for PRS and a change in the

Table IV. Inhibition of ATP-PP_i Exchange by α-Substituted Phenylethylamines[a]

	C_6H_5-CH_2CH(CH_2)$_n$-C_6H_5 NHR			
	R	n	$(I)_{0.5}$, mM[b]	K_i, μM
23	H	0	1.7	280
24	H	1	0.2	33
25	H	2	0.12	20
26	$CH_2C_6H_5$	0	c	d
27	$CH_2C_6H_5$	1	0.024[f]	e
28	$CH_2C_6H_5$	2	0.06	d

[a] Compounds 23, 25, 26, and 28 were racemic mixtures. [b] Concentration of inhibitor to obtain 50% inhibition at 0.3 mM L-phenylalanine. [c] No inhibition at 0.1 mM (maximum solubility). [d] Too insoluble for assay. [e] Noncompetitive inhibition. [f] Value estimated by extrapolation from concentration of 8 μM required for 25% inhibition.

mode of inhibition: *N*-phenylethyl- (**29**), *N*-phenylpropyl- (**30**), and *N*-phenylbutyl-2-phenylethylamines (**31**) are all noncompetitive inhibitors of this enzyme. Similarly, substitution at the α position of the *N*-benzyl moiety of *N*-benzyl-2-phenylethylamine (**7**) results in a decreased affinity for PRS. The α-methyl derivative **32** is a competitive inhibitor with K_i = 10 μM but the α-ethyl (**33**), α-propyl (**34**), α-isopropyl (**35**), α-phenyl (**36**), and α-benzyl (**37**) derivatives are noncompetitive inhibitors with respect to L-phenylalanine. Alkylation of the amino group of 2-phenylethylamine derivatives to give *N*-methyl-*N*-benzyl-2-phenylethylamine (**38**), *N*,*N*-dibenzyl-2-phenylethylamine (**41**), and *N*-methyl-*N*-benzyl-D- and -L-amphetamine (**40**, **39**) also resulted in poor noncompetitive inhibitors of PRS.

Substitution at the α position of 2-phenylethylamine (**1**) by benzyl and 2-phenylethyl substituents gave **24** and **25** with K_i values of 33 and 20 μM, respectively; the α-phenyl derivative **23** had K_i = 280 μM as shown in Table IV. Unfortunately, addition of *N*-benzyl groups to these derivatives to give **26**, **27**, and **28** (Table IV) resulted in compounds of limited solubilities which could not be accurately assessed for their inhibitory properties; however, it was apparent that addition of the *N*-benzyl groups did not result in the large enhancements in affinity observed for similar modification of 2-phenylethylamine (**1**), D-amphetamine (**5**), or D-phenylalaninol (**3**).

Discussion

Previous reports from this laboratory described the design and evaluation of a large number of inhibitors of PRS-catalyzed ATP-PP_i exchange.[2,3] In one such study of analogues of L-phenylalanine,[2] it was observed that 2-phenylethylamine (**1**) was a good competitive inhibitor ($K_i \simeq 0.1$ mM) and its potency could be increased (~1.5 kcal/mol) by placement of a methyl (L-amphetamine) or hydroxymethyl (L-phenylalaninol) group in the 1 position, occupying the same configuration as the carboxylate moiety of L-phenylalanine. This and related findings led to the conclusion that the area of the enzyme juxtapositioned to the carboxyl moiety of L-phenylalanine was partially hydrophobic. Furthermore, a high degree of stereospecificity was observed in binding of these analogues to PRS. For example, D-phenylalaninol (**3**) and D-amphetamine (**5**), which have the same substituents as mentioned above, but in the position occupied by the α-hydrogen of L-phenylalanine, showed losses of 2–3 kcal/mol in binding energy compared to the L isomers. This observation is in accord with the poor binding of D-phenylalanine to PRS and indicates a rigid steric requirement in this area of the enzyme active site.

From an examination of L-phenylalanine analogues in which the α-amino group was omitted, substituted for by other groups, or modified, we were led to believe that the unsubstituted amino group of L-phenylalanine and related analogues was essential for binding and that modifications of this group could not be made without large losses in binding. For example, *N*-methyl-2-phenylethylamine and *N*-methylamphetamine bind ~2–3 kcal/mol poorer than the parent unsubstituted amines.[2] In the present work, we have demonstrated that addition of an *N*-benzyl group to 2-phenylethylamine results in a 3 kcal/mol *increase* in binding affinity compared to 2-phenylethylamine (**1**), suggesting that a previously undiscovered hydrophobic area exists near the binding locus for L-phenylalanine. From structure–binding relationships we rationalized that since L-phenylalaninol (**2**) and L-amphetamine (**4**) bind significantly better than 2-phenylethylamine (**1**), the corresponding *N*-benzyl derivatives should be extremely potent inhibitors with K_i values less than 0.1 μM. As is often the case in such studies, experimentation did not support hypothesis: *N*-benzyl-L-phenylalaninol (**8**) and *N*-benzyl-L-amphetamine (**10**) were found to inhibit PRS to about the same extent as the parent unsubstituted amines. Most surprising was the finding that *N*-benzyl-D-amphetamine (**11**) and *N*-benzyl-D-phenylalaninol (**9**) were extremely potent inhibitors of PRS. The addition of an *N*-benzyl substituent increases the binding affinity of these rather poor inhibitors of PRS by over 4 kcal/mol. In fact, *N*-benzyl-D-amphetamine (**11**) is the most potent inhibitor of PRS yet described with a K_i = 0.14 μM. Thus, there is an interesting discrepancy in structure–activity relationships in that L-phenylalaninol (**2**) and L-amphetamine (**4**) bind much tighter than their D isomers, whereas in the *N*-benzyl series, the D isomers are the more potent inhibitors.

One explanation for this discrepancy is that *N*-benzyl-2-phenylethylamine (**7**) and derivatives may have two modes of binding to PRS. For example, the tight binding of *N*-benzyl-D-amphetamine (**11**) and *N*-benzyl-D-phenylalaninol (**9**) may result from the *N*-benzyl group occupying the previously described[2] binding site for the phenyl ring of L-phenylalanine, L-amphetamine, or L-phenylalaninol. In this manner, the unfavorable steric interactions which would result from projection of the methyl or hydroxymethyl substituents into the sterically restricted region of PRS juxtaposed to the α-hydrogen of L-phenylalanine would be avoided, and the increased affinity of the D isomers might be ascribed to favorable interactions of the α-substituents with as yet unmapped regions of the enzyme.

Although this alternative or dual mode of binding is very difficult to verify experimentally, an approach has been attempted to gain some insight into the feasibility of this hypothesis. We have previously demonstrated that the binding site of PRS for the phenyl ring of L-phenylalanine and analogues such as 2-phenylethylamine (**1**) is extremely intolerant to substituents.[2] This is not surprising when one considers the specificity required of an aminoacyl-tRNA synthetase to recognize only its cognate amino acid. Thus, placement of a substituent on the phenyl ring of the phenylethyl moiety of *N*-benzyl-2-phenylethylamine (**7**) would be expected to result in a decrease in binding affinity analogous to that observed with the corresponding primary amines. However, if the substitution is sufficiently unfavorable and the second hydrophobic area will tolerate such substituents, then the *N*-benzyl group might bind in the parent phenyl site and the substituted ring in the

second hydrophobic area; in this manner the expected detrimental effect of the substitution could be compensated for. However, if the same substituent is placed on both phenyl rings of *N*-benzyl-2-phenylethylamine (7), the option of alternative or dual binding would not be available and the compound should be a poorer inhibitor than either of the monosubstituted compounds.

We have attempted this approach with *p*-hydroxyl- and *o*-methyl-substituted analogues of *N*-benzyl-2-phenylethylamine. The *p*-hydroxyl substituent causes large losses (~3 kcal/mol) in binding for each of the monosubstituted compounds (13 and 15) so it is not well suited for the purpose described here; nevertheless *N*-(*p*-hydroxybenzyl)tyramine (14), the analogue with *p*-hydroxyl substituents on both rings, binds ~1 kcal/mol poorer than either of the monosubstituted compounds and lends some support to the aforementioned hypothesis. The *o*-methyl-substituted *N*-benzyl-2-phenylethylamines were somewhat more informative in supporting the hypothesis of alternate or dual modes of binding for these analogues. As with the *p*-hydroxyl group, addition of an *o*-methyl group to phenylalanine, phenylalaninol, amphetamine, or 2-phenylethylamine leads to large losses in binding affinity for PRS.[2] However, in contrast with the monosubstituted *p*-hydroxyl analogues of *N*-benzyl-2-phenylethylamine, placement of an *o*-methyl group on either of the rings of *N*-benzyl-2-phenylethylamine (7) provides analogues (16 and 18) which bind about the same as the parent compound (7). It is logical to propose that the unsubstituted ring in each of these analogues may interact with the binding site on PRS which normally complexes the phenyl ring of L-phenylalanine and related analogues. When the *o*-methyl group is placed on both rings (17), a loss in binding affinity is incurred as expected from the hypothesis for alternative, or dual, binding modes. Unfortunately, the losses are not as great as those observed for the primary amines mentioned above and if our hypothesis is correct, other unrecognized secondary binding effects must play a role in the binding of the substituted *N*-benzyl-2-phenylethylamine analogues described here.

To the extent that they were examined, other substituents on the *N*-benzyl group of *N*-benzyl-2-phenylethylamine (7) had varied effects on binding to PRS depending upon the position and nature of the substituent. As with the *o*-methyl group, a methoxyl substituent in the ortho position (21) had little effect on the inhibitory potency as compared to *N*-benzyl-2-phenylethylamine (7), but a nitro substituent in the same position (22) resulted in ~2 kcal/mol decrease in binding. Substitutions with methyl groups at the meta (19) and para (20) positions of 7 were similarly detrimental to binding. Although the number of *N*-benzyl substituents examined was not sufficient to formulate accurate structure–binding relationships, this region is clearly one which should be examined further in studies aimed at designing more potent reversible inhibitors and active-site-directed irreversible inhibitors of PRS.

A number of additional structural modifications of *N*-benzyl-2-phenylethylamine were made in search of new avenues for increasing the inhibitory potency of the prototype analogue. As described elsewhere in detail,[2] an additional hydrophobic region of PRS appears to exist adjacent to the somewhat hydrophobic carboxylate binding region of the enzyme. We have examined the inhibitory properties of a number of 2-phenylethylamine derivatives with α-hydrophobic substituents larger than the methyl group of amphetamine, with the hope that these substituents might provide additional interactions with the aforementioned adjacent hydrophobic region of PRS. Placement of a 1-phenyl group on 2-phenylethylamine to give 1,2-diphenylethylamine (23) resulted in a small loss in binding. However, the 1-benzyl (24) and 1-phenylethyl (25) substituted analogues were more potent inhibitors than 2-phenylethylamine (1) and bound approximately as well as L-amphetamine (4). Although the potency of these inhibitors falls short of what we had hoped for, because of the ease and variability with which the phenyl ring may be modified, they do provide an attractive avenue for future design of irreversible inhibitors. Using the additional interactions achieved by this approach, the addition of an *N*-benzyl group to such inhibitors should, in theory, have resulted in extremely potent inhibition of PRS. Unfortunately, the corresponding *N*-benzyl analogues (26, 27, 28) were either too water insoluble to assay or displayed noncompetitive inhibition with respect to phenylalanine.

Addition of substituents at the α position of the *N*-benzyl moiety of *N*-benzyl-2-phenylethylamine (7) gave a series of compounds (32–37, Table V) showing only moderate to poor noncompetitive inhibition of PRS-catalyzed ATP–PP_i exchange. The α-methyl derivative *N*-(1-phenylethyl)-2-phenylethylamine (32), the only competitive inhibitor observed, showed a loss of ~1.7 kcal/mol in binding affinity with respect to *N*-benzyl-2-phenylethylamine (7), implying that a lack of tolerance to bulk exists in this region of the binding site.

Further extension of the *N*-benzyl moiety of *N*-benzyl-2-phenylethylamine by one (29), two (30), or three (31) methylene groups (Table V) gave a series of poor noncompetitive inhibitors which had $(I)_{0.5}$ values 30–100-fold higher than *N*-benzyl-2-phenylethylamine (7). Alkylation of the amino group of *N*-benzyl-2-phenylethylamine as shown in Table VI gave poor noncompetitive inhibitors which showed 6–40-fold losses in binding power as indicated by $(I)_{0.5}$ values. N-Methylation of *N*-benzyl-L-amphetamine and *N*-benzyl-D-amphetamine also

Table V. Noncompetitive Inhibition of ATP–PP_i Exchange by C_6H_5-CH_2CH_2NH·HCl (R$_2$)

No.	R_2	$(I)_{0.5}$, μM[a]
29	$-CH_2CH_2C_6H_5$	118
30	$-CH_2(CH_2)_2C_6H_5$	330
31	$-CH_2(CH_2)_3C_6H_5$	390
32	$-CH(C_6H_5)CH_3$	140[b,c]
33	$-CH(C_6H_5)CH_2CH_3$	1220[c]
34	$-CH(C_6H_5)CH_2CH_2CH_3$	1020[c]
35	$-CH(C_6H_5)CH(CH_3)_2$	3650[c]
36	$-CH(C_6H_5)_2$	~160[d]
37	$-CH(C_6H_5)CH_2C_6H_5$	~160[c,d]

[a] Inhibitor concentration required for 50% inhibition of ATP–PP_i exchange at 0.3 mM L-phenylalanine. [b] Competitive inhibitor K_i = 10 μM. [c] Racemic mixture. [d] Value estimated by extrapolation from concentration (95 μM) required for 20% inhibition.

Table VI. Noncompetitive Inhibition of ATP–PP_i Exchange by C_6H_5-$CH_2CH(R_1)NCH_2$-C_6H_5 (R_2)

No.	R_1	R_2	$(I)_{0.5}$, μM
38	-H	$-CH_3$	24
39	$-CH_3$ (L)	$-CH_3$	450
40	$-CH_3$ (D)	$-CH_3$	155
41	-H	$-CH_2C_6H_5$	160

gave poor noncompetitive inhibitors (Table V) which showed 13- and 190-fold losses, respectively, in inhibitory potency as indicated by $(I)_{0.5}$ values.

Omission of the α-carboxylate group of amino acids or replacement by small nonpolar groups generally provides good inhibitors of the corresponding aminoacyl-tRNA synthetase.[1,2,4,6] Thus, as 2-phenylethylamine and amphetamine bind tightly to PRS, tyramine and *p*-hydroxyamphetamine are potent inhibitors of *E. coli* tyrosyl-tRNA synthetase,[4] and isobutylamine is a good inhibitor of *E. coli* valyl-tRNA synthetase.[6] In contrast to the high affinity for PRS of the *N*-benzyl derivatives described herein, N-benzylation of the aforementioned tyrosine and valine analogues results in large (150–200-fold) decreases in affinity for the corresponding synthetases[4] (D. V. Santi, unpublished results). These limited observations suggest that the large increase in affinity obtained from the *N*-benzyl group may be a unique feature with PRS; it is clearly unwarranted at this time to assume that N-benzylation of amino acid analogues might be of general utility in designing inhibitors for other aminoacyl-tRNA synthetases.

In summary, the present work describes a potent new class of inhibitors for *E. coli* PRS. The prototype, *N*-benzyl-2-phenylethylamine, appears to possess the structural features required for near-optimal binding. Certain structural modifications of the prototype, such as conversion of the secondary nitrogen to tertiary, alkyl substitution at the α position of the *N*-benzyl moiety, or further separation of the phenyl ring from the nitrogen, resulted in inhibitors noncompetitive with respect to L-phenylalanine. These were invariably poorer inhibitors than the prototype. In contrast, when substituents were placed on either of the phenyl rings, competitive inhibitors with respect to L-phenylalanine were obtained and showed large variations in inhibitory power. From the limited number of compounds studied in this series, it appears that substituents at the ortho position of either ring are well tolerated and worthy of further investigation. Substitution at both phenyl rings of the prototype provides competitive inhibitors which are less potent than the monosubstituted derivatives. Most interesting was the finding that methyl substitution for the α-hydrogen of the 2-phenylethylamine moiety results in the most potent competitive inhibitor yet described for PRS from *E. coli*, *N*-benzyl-D-amphetamine ($K_i = 0.14\ \mu M$). In view of the fact that this analogue binds to PRS with the same affinity[9] as Phe-tRNA, is highly stereoselective in binding, and has a K_i value lower than the estimated concentration of aminoacyl-tRNA synthetases present in bacterial cells, we were tempted to anticipate that *N*-benzyl-D-amphetamine (11) might act as a selective inhibitor of PRS, and thus protein biosynthesis in vivo. Studies of this aspect have demonstrated that this is indeed the case, and a complete report of in vivo effects of this analogue is forthcoming. In addition, the accompanying report[29] demonstrates that analogues of *N*-benzyl-2-phenylethylamine are remarkably species selective in that they completely inhibit *E. coli* PRS at concentrations which have no effect on PRS from rat liver.

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