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Conjugates of Catecholamines. 5. Synthesis and β-Adrenergic Activity of *N*-(Aminoalkyl)norepinephrine Derivatives[1]

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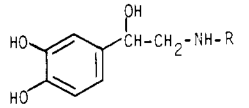
A novel series of *N*-aminoalkyl congeners and model derivatives of norepinephrine has been synthesized. Compounds that were structurally related to epinephrine were prepared from fully protected intermediates. Alternatively, isoproterenol-related compounds were synthesized via reductive amination of preformed methyl ketone derivatives with norepinephrine. The β-adrenergic activities of these new compounds were assessed through measurement of intracellular cyclic AMP accumulation in S49 mouse lymphoma cells and displacement of iodocyanopindalol (ICYP) from membrane preparations. Congeners that contained an underivatized primary amine function exhibited virtually no activity in these assays. However, when this amine function was acylated (e.g., to an amide, carbamate, urea, sulfonamide, etc.), the products exhibited generally increased β-adrenergic activity, which was, however, strongly dependent on the nature of the acylating group and also the length of the spacer. In particular, a benzyl carbamate derivative containing a branched, seven-carbon spacer group was 40 times more potent than isoproterenol in the in vitro S49 assay.

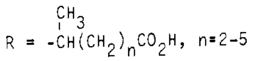
We have pursued a systematic investigation to develop a variety of approaches to linkages between carriers such as peptides and the β-adrenergic drug isoproterenol (1).[1] We have prepared functionalized catecholamines, termed congeners, and model compounds of specific peptide–drug linkages. A number of these model compounds have shown unexpected and potentially useful pharmacologic activity of their own, and they augment the structure–activity relationship (SAR) analysis of catecholamines based on previously prepared compounds.



1

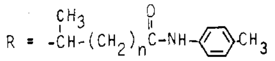
1 $R = -CH(CH_3)_2$

2



$R = -CH(CH_3)(CH_2)_nCO_2H$, n=2-5

3



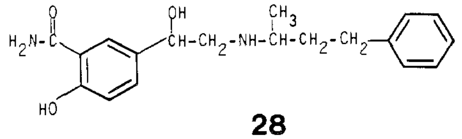
$R = -CH(CH_3)-(CH_2)_n-C(=O)-NH-C_6H_4-CH_3$

n=2-5

We first examined derivatization of the aromatic ring of isoproterenol (1). Direct substitution on the ring, however, in most cases eliminated β-adrenergic activity, making this approach unattractive for attachment to carriers.[1a,b] Substitution by an ether linkage to the benzylic hydroxyl has been reported to result in the loss of activity.[4] When we prepared and rigorously purified the β-ethyl ether derivative of isoproterenol, we also observed virtually complete loss of biological activity.

Subsequently, we developed a novel approach in which the isopropyl group of isoproterenol was extended by an alkyl chain terminating in a functional group, specifically designed for attachment to a carrier. Already reported are the synthesis and biological activity of a series of carboxylic acid congeners 2 and the amide model compounds 3.[1c] In this series, the biological activity of the model amides 3 was strikingly dependent on both the length of the spacer and also the nature of the amide substituent.[1c–e] In general, maximal activity was observed for compounds containing a six-carbon, branched spacer (**3**, *n* = 4) and, depending on the amide substituent, both in vitro[1c] and in vivo[1d,e] potencies were often much greater than that of isoproterenol.

We have explored the use of several other functionalities in the *N*-alkyl side chain and here report the synthesis and activity of a series of new congeners and model derivatives. These new compounds are based on the *N*-(aminoalkyl)norepinephrine congeners 4 and 8–11 (Table I) and include model derivatives prepared by conversion of the primary amine into acylated derivatives such as amides, urethanes, ureas, or sulfonamides (compounds 5–7 and 12–27, Table I). A model dipeptide conjugate 27 has also been prepared. Several compounds related to epinephrine have been prepared recently that are similar in structure to some of those presented here.[6] Also worthy of note are mixed α- and β-blocking drugs such as labetalol (**28**), a cardioselective hypotensive agent that bears an amide functionality on the aromatic ring.[7]



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Synthesis of Congeners and Model Derivatives

Two distinct synthetic routes have been used for the

[†] University of California, San Diego.

[‡] Stanford University Medical Center.

[§] University of California, San Francisco.

(1) For previous papers in this series, see: (a) Avery, M. A.; Verlander, M. S.; Goodman, M. *J. Org. Chem.* **1980**, *45*, 2750–2753; 1981, *46*, 5459. (b) Reitz, A. B.; Avery, M. A.; Verlander, M. S.; Goodman, M. *J. Org. Chem.* 1981, *46*, 4859–4863. (c) Jacobson, K. A.; Marr-Leisy, D.; Rosenkranz, R. P.; Verlander, M. S.; Melmon, K. L.; Goodman, M. *J. Med. Chem.* **1983**, *26*, 492-499. (d) Verlander, M. S.; Jacobson, K A.; Rosenkranz, R. P.; Melmon, K. L.; Goodman, M. *Biopolymers* 1983, *22*, 531–545. (e) Rosenkranz, R. P.; Hoffman, B. B.; Jacobson, K. A.; Verlander, M. S.; Klevans, L.; O'Donnell, M.; Goodman, M.; Melmon, K. L. *Mol. Pharmacol.* 1983, *24*, 429–435. (f) Rosenkranz, R. P.; Jacobson, K. A.; Verlander, M. S.; Klevans, L.; O'Donnell, M.; Goodman, M.; Melmon, K. L. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 267–273.

(2) Philips, D. *Handb. Exp. Pharm.* **1980**, *54/I*, 3–63.

(3) Triggle, D. J. "Medicinal Chemistry"; Burger, A., Ed.; New York, 1980; Part III, pp 225–284.

(4) See ref 26 of Jen, T.; Frazee, J.; Schwartz, M.; Erhard, K.; Kaiser, C.; Colella, D.; Wardell, J., Jr. *J. Med. Chem.* **1977**, *20*, 1263–1268.

(5) Sonveaux, E.; Verlander, M. S.; Goodman, M.; Melmon, K. L., unpublished results.

(6) Barlow, J.; Main, B.; Snow, H. *J. Med. Chem.* **1981**, *24*, 315–322.

(7) Comer, W. T.; Matier, W. L.; Amer, M. S. "Medicinal Chemistry"; Burger, A., Ed.; New York, 1980; Part III, pp 286–337.

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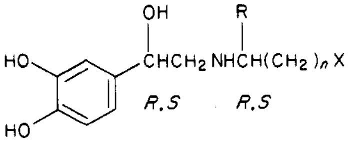
Table I. *N*-(Aminoalkyl)norepinephrine Derivatives



compd	R	*n*	X	rel[a] potency	yield,[b] %	method	formula
4	H	2	NH_2	2×10^{-4}	50	A	$C_{11}H_{18}N_2O_3 \cdot 2HCl$
5	H	2	NHCONHPh	1.9×10^{-2}	43	A	$C_{18}H_{23}N_3O_4 \cdot HCl$
6	H	2	$NHCOCH_3$	2.1×10^{-3}	80	A	$C_{13}H_{20}N_2O_4 \cdot H_3PO_4$
7	H	2	$NHCOCH_2NHCOCH_3$	1.16×10^{-5}	50	A	$C_{15}H_{23}N_3O_5 \cdot H_3PO_4$
8	CH_3	2	NH_2	2.0×10^{-7}	25	D	$C_{12}H_{20}N_2O_3 \cdot 2HCl$
9	CH_3	3	NH_2	1.1×10^{-7}	32	D	$C_{13}H_{22}N_2O_3 \cdot 2HCl$
10	CH_3	4	NH_2	3×10^{-4}	50	E	$C_{14}H_{24}N_2O_3 \cdot 2HCl$
11	CH_3	5	NH_2	0.27×10^{-7}	31	E	$C_{15}H_{26}N_2O_3 \cdot 2HCl$
12	CH_3	2	$NHCOOCH_2Ph$	0.61	47	B	$C_{19}H_{24}N_2O_5 \cdot HCl$
13	CH_3	3	$NHCOOCH_2Ph$	0.72	79	B	$C_{20}H_{26}N_2O_5 \cdot HCl$
14	CH_3	4	$NHCOOCH_2Ph$	1.67	32	B	$C_{21}H_{28}N_2O_5 \cdot HCl$
15	CH_3	5	$NHCOOCH_2Ph$	5.5	55	B	$C_{22}H_{30}N_2O_5 \cdot HCl$
16	CH_3	3	$NHCOO(c\text{-}C_6H_{11})$	1.0	90	B	$C_{20}H_{32}N_2O_5 \cdot HCl$
17	CH_3	2	$NHCO(C_6H_4)\text{-}4\text{-}Me$	0.72×10^{-5}	86	B	$C_{20}H_{26}N_2O_4 \cdot HCl$
18	CH_3	3	$NHCO(C_6H_4)\text{-}4\text{-}Me$	0.19	44	B	$C_{21}H_{28}N_2O_4 \cdot HCl$
19	CH_3	4	$NHCO(C_6H_4)\text{-}4\text{-}Me$	0.89	33	B	$C_{22}H_{30}N_2O_4 \cdot HCl$
20	CH_3	5	$NHCO(C_6H_4)\text{-}4\text{-}Me$	3.6×10^{-4}	58	B	$C_{23}H_{32}N_2O_4 \cdot HCl$
21	CH_3	2	$NHCO(C_6H_4)\text{-}2\text{-}Me$	1.2×10^{-5}	84	B	$C_{20}H_{26}N_2O_4 \cdot HCl$
22	CH_3	3	$NHCO(C_6H_4)\text{-}2\text{-}Me$	3.6×10^{-6}	46	B	$C_{21}H_{28}N_2O_4 \cdot HCl$
23	CH_3	4	$NHCO(C_6H_4)\text{-}2\text{-}Me$	3.9×10^{-9}	54	B	$C_{22}H_{30}N_2O_4 \cdot HCl$
24	CH_3	5	$NHCO(C_6H_4)\text{-}2\text{-}Me$	0.65×10^{-5}	60	B	$C_{23}H_{32}N_2O_4 \cdot HCl$
25	CH_3	2	$NHSO_2(C_6H_4)\text{-}4\text{-}Me$	0.73	73	C	$C_{19}H_{26}N_2O_5S\text{-}HCl$
26	CH_3	4	$NHCONH(C_6H_4)\text{-}4\text{-}Me$	0.13	37	B	$C_{22}H_{31}N_3O_4 \cdot HCl$
27	CH_3	4	$NHCONH(Phe(Ac)GlyNHCH_3)$	9.83×10^{-6}	25	B	$C_{28}H_{42}N_6O_7 \cdot HCl$

[a] Determined by measuring the accumulation of cyclic AMP in SF49 mouse lymphoma cells relative to *dl*-isoproterenol.[1,c] The K_A values for isoproterenol and the test compounds were determined from a minimum of eight different concentrations ranging from 10^{-5} to 10^{-12} M. Each K_A value was derived from at least three determinations each in triplicate. The ratios did not vary ($p < 0.05$) between experiments. Eight concentrations of propranolol were used in blocking experiments (ranges from 10^{-5} to 10^{-12} M). Each test compound was used at the concentration that produced its maximal efficacy in cyclic AMP generation in S49 cells. All points were the mean of triplicate experiments whose coefficient of variability was less than 10%. The K_D of selected compounds for displacement of ICYP binding are as follows ($\bar{X} \pm$ SEM): isoproterenol, $9.8 \times 10^{-7} \pm 2.4 \times 10^{-8}$; **4**, $1.6 \times 10^{-6} \pm 8.8 \times 10^{-6}$; **10**, $1.0 \times 10^{-5} \pm 2.2 \times 10^{-7}$; **14**, $4.9 \times 10^{-8} \pm 7.1 \times 10^{-10}$; **15**, $1.2 \times 10^{-7} \pm 1.5 \times 10^{-9}$; **19**, $3.2 \times 10^{-7} \pm 4.0 \times 10^{-9}$; **26**, $4.7 \times 10^{-7} \pm 4.5 \times 10^{-9}$. [b] Isolated yields.

synthesis of the congeners and model derivatives. The epinephrine-related derivatives **4–7** were prepared as shown in Scheme I. Reaction of *N*-benzyl-3-chloropropylamine (**29**) with potassium phthalimide gave *N*-benzyl-3-phthalimidopropylamine (**30**). This amine was allowed to react with bromoacetophenone **31** to yield compound **32** in high yield. Reduction of the ketone with aluminum isopropoxide produced the alcohol **33**. Subsequent removal of the phthalimido group with hydrazine gave compound **34**. Deprotection by catalytic hydrogenation yielded the diamine **4**. Alternatively, prior to deprotection, the primary amine of compound **34** was acylated to give the intermediates **36** and **37** or treated with phenyl isocyanate to form the urea **35**. Removal of the benzyl groups then produced the congener model compounds **5–7**.

The isoproterenol derivatives **8–27** were prepared by a somewhat different route utilizing reductive amination of norepinephrine **38** with the appropriate methyl ketone as the final synthetic step.[1c] Thus, a series of keto carboxylic acids was subjected to a Curtius rearrangement using diphenylphosphoryl azide (DPPA)[9] as shown in Scheme II. The resulting keto isocyanates were not isolated but were allowed to react with benzyl alcohol to give the keto carbamates **39–42** in yields of 49–57%. Compounds **39–42** underwent reductive amination with *dl*-norepinephrine hydrochloride (**38**) with use of sodium cyanoborohydride to give the catecholamine derivatives **12–15**, which were purified on a small scale for biological testing or carried on to the next step. Removal of the benzyloxycarboxyl groups of compounds **12–15** generated the diamines **8–11**. Alternatively, the keto isocyanates were reacted with N^{α}-acetyl-*p*-amino-L-phenylalanylglycine benzyl ester, *p*-toluidine, or cyclohexanol to yield the methyl ketones **47–49**, respectively (Scheme III). Compounds **48** and **49** were reacted with norepinephrine (**38**) directly to give the model compounds **26** and **16**. Compound **47** was converted to its *N*-methyl amide **50**, which then underwent reductive amination with norepinephrine (**38**) to produce the dipeptide conjugate **27**.

The intermediate keto isocyanates were also treated with aluminum chloride in toluene to produce mixtures of *o*- and *p*-methyl keto amides **43–46**, which could not be separated easily by crystallization or chromatography (Scheme II). The total yield of amide products in the reaction was low (15–31% based on the starting carboxylic acids); however, the yields were not optimized. *p*-Tolyl products predominated (62–84%), and the proportion of ortho isomer decreased as the ketone carbonyl became more distant from the isocyanate functionality. The relative amounts of ortho and para products were determined by integrating the separate amide proton resonances in the 360-MHz ^1H NMR spectra, and the aromatic resonances showed a superposition of the expected splitting patterns for the two isomers. The electrophilic addition of alkyl isocyanates to toluene mediated by aluminum chloride is reported to proceed with virtually complete para orientation.[10] The unexpectedly high proportion of ortho product

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(8) (a) Coffino, P.; Bourne, H. R.; Insel, P. A.; Melmon, K. L.; Johnson, G.; Vigne, J. *In Vitro* 1978, *14*, 140–145. (b) Gilman, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 305–312.

(9) Ninomiya, N.; Shiori, T.; Yamada, S. *Tetrahedron* 1974, *30*, 2151–2157.

Scheme I

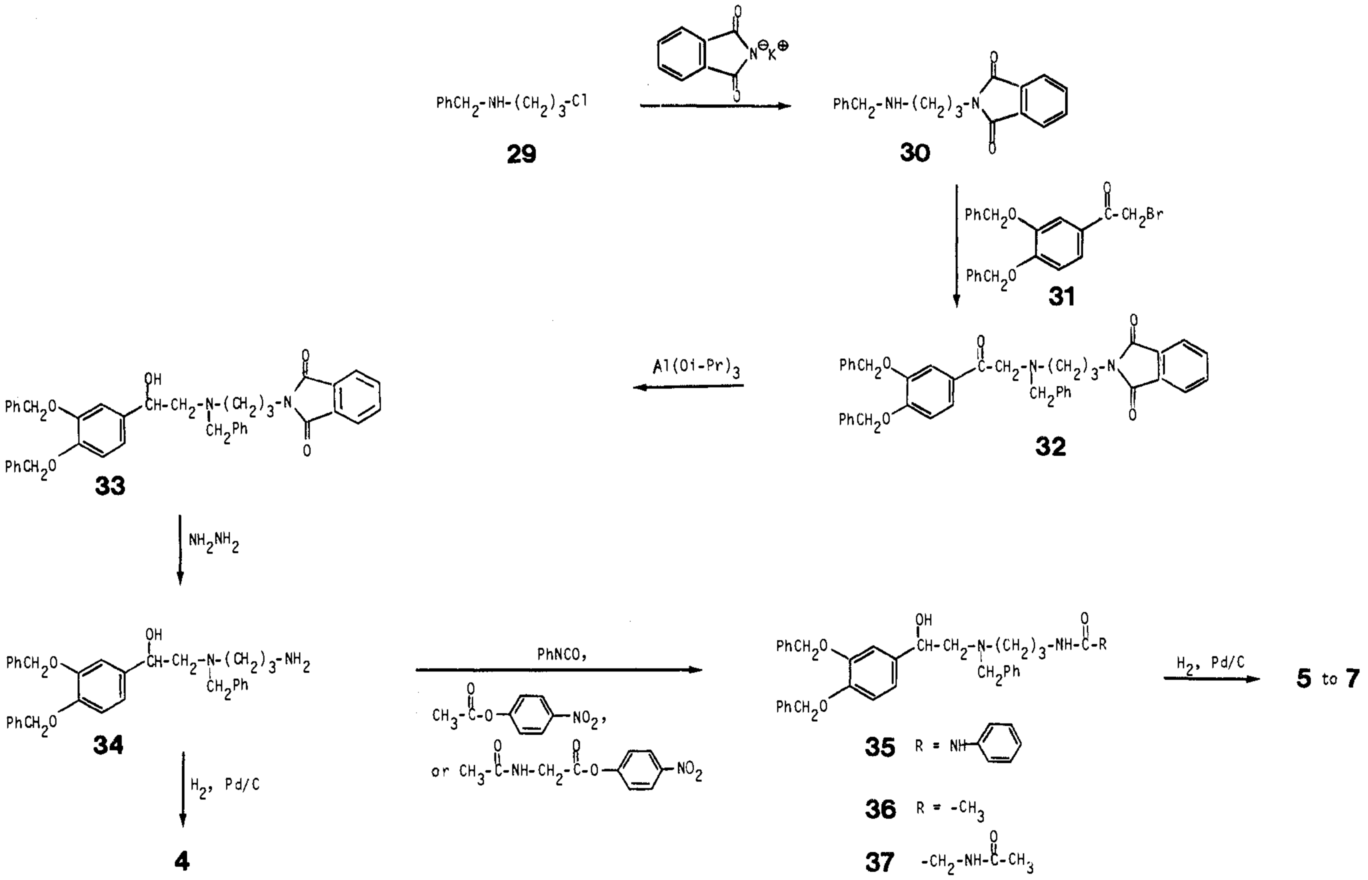
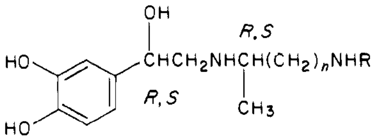


Table II. Protonated Molecular and Fragment Ions of (Alkylamino)norepinephrine Derivative Observed under LSI Mass Spectral Conditions



compd	*n*	R	MH$^+$	i	ii	iii	iv
14	4	$COOCH_2Ph$	403	387	385	263	251
16	3	$COO(c\text{-}C_6H_{11})$	381	365	363	241	229
20	5	$CO(C_6H_4)\text{-}4\text{-}Me$	401	385	383	261	249
25	2	$SO_2(C_6H_4)\text{-}4\text{-}Me$	395	379	377	255	243
26	4	$CONH(C_6H_4)\text{-}4\text{-}Me$	402	386	384	262	250

in compounds **43–46** may be due to an activating effect of the carbonyl group making the isocyanate more reactive and less selective.[11] Upon reductive amination with norepinephrine, the ortho and para isomers derived from compounds **43–46** could be separated cleanly by reverse phase HPLC,[13,14] yielding the derivatives **17–20** and **21–24**. The ortho isomers, clearly recognized by their characteristic splitting in the aromatic region of the NMR spectra, eluted faster on the reverse-phase HPLC columns than the corresponding para isomers.

The keto sulfonamide **51** was prepared by the addition of *p*-toluenesulfonamide to methyl vinyl ketone mediated by alumina[13-15] (Scheme IV). Compound **51** underwent reductive amination with norepinephrine to yield the catecholamine derivative **25** (Scheme IV).

Compounds **4–27** were first subjected to an extractive workup and then rigorously purified by reversed-phase semipreparative high-performance chromatography by use of literature procedures.[1c,14] All of the methyl ketones used in the reductive amination were characterized by elemental analysis and standard spectroscopic techniques (see Experimental Section). Compounds **4–27** were characterized by a combination of 360-MHz ^1H NMR and HPLC (>99%), and additionally, several of these compounds were

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(10) Effenberger, F.; Gleiter, R. *Chem. Ber.* **1964**, *97*, 472–479.

(11) The reaction of aryl isocyanates (which are more reactive than alkyl isocyanates[12]) with toluene mediated by Lewis acid catalysts gives significant amounts of ortho substitution. Alder, R.; Chalkley, G.; Whiting, M. *J. Chem. Soc.* **1966**, 52–53.

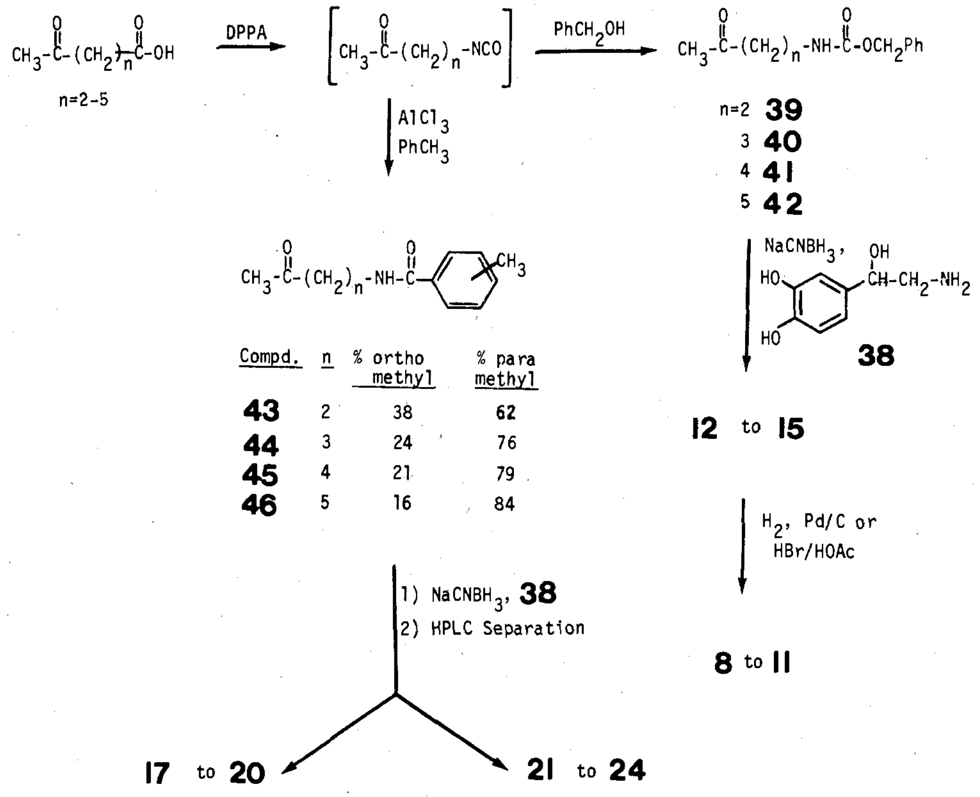
(12) Saunders, J. "Polyurethanes, Chemistry and Technology"; Frisch, K., Ed.; Interscience: New York, 1962; Part I.

(13) (a) Supplementary material details the HPLC parameters and ^1H NMR data of the final products 4–27. (b) Reitz, A. B. Ph.D. Thesis, University of California, San Diego, 1982.

(14) (a) Molnar, I.; Horvath, C. *Clin. Chem.* **1976**, *22* 1497–1502. (b) Scratchley, G. A.; Masoud, A. N.; Strohs, S. J.; Wingard, D. W. *Chromatographia* **1979**, *17*, 279–309. (c) Krstulovic, A. M. *Adv. Chromatogr.* **1979**, *17*, 279–309.

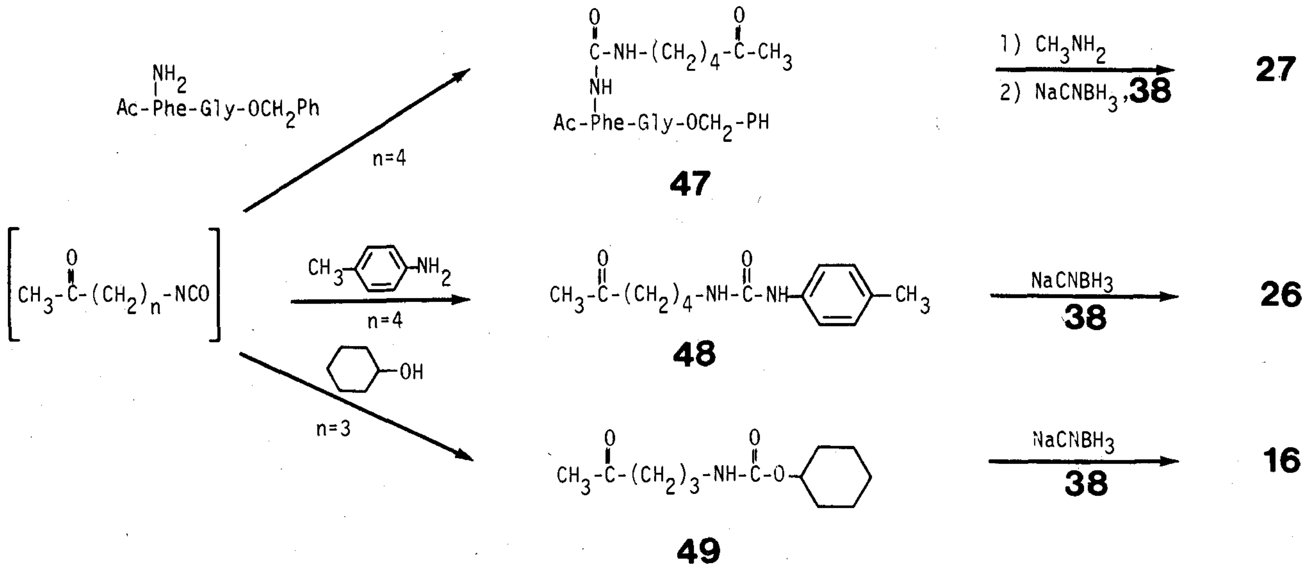
(15) Reitz, A.; Verlander, M.; Goodman, M. *Tetrahedron Lett.* **1982**, *23*, 751–752.

Scheme II

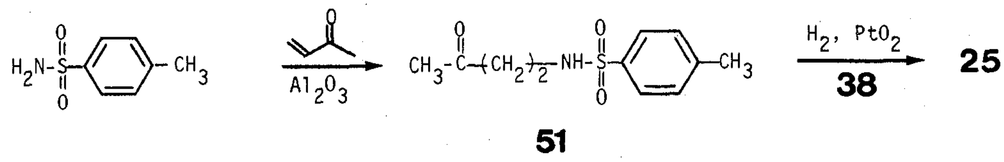


Compd.	n	% ortho methyl	% para methyl
43	2	38	62
44	3	24	76
45	4	21	79
46	5	16	84

Scheme III



Scheme IV



examined by liquid secondary ion mass spectrometry (LSIMS),[16] a technique that has proven extremely powerful in the characterization of a variety of polar molecules.

Figure 1 shows the LSI mass spectrum of the sulfonamide derivative **25**. The spectrum is typical of those obtained with this series of compounds. An intense protonated molecular ion (in this case, the base peak) appears at mass 395. Fragment ions at *m*/*e* 379, 377, 243, 241, 155, and 152 are readily assigned to the species shown in the figure. The ion marked "gly" are well-established species due to the presence of protonated glycerol (MH^+ 93), protonated glycerol dimer (MH^+ 185), and protonated glycerol trimer (MH^+ 277).

The mass fragmentation patterns (Scheme V) of the series of compounds examined were remarkably similar

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[16] Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029–2034.

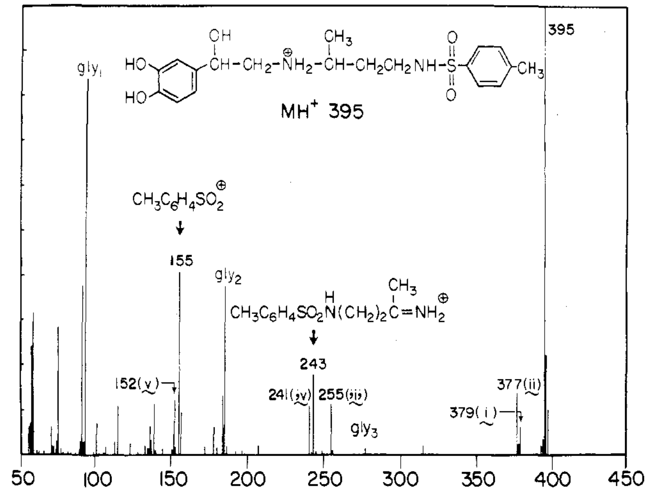
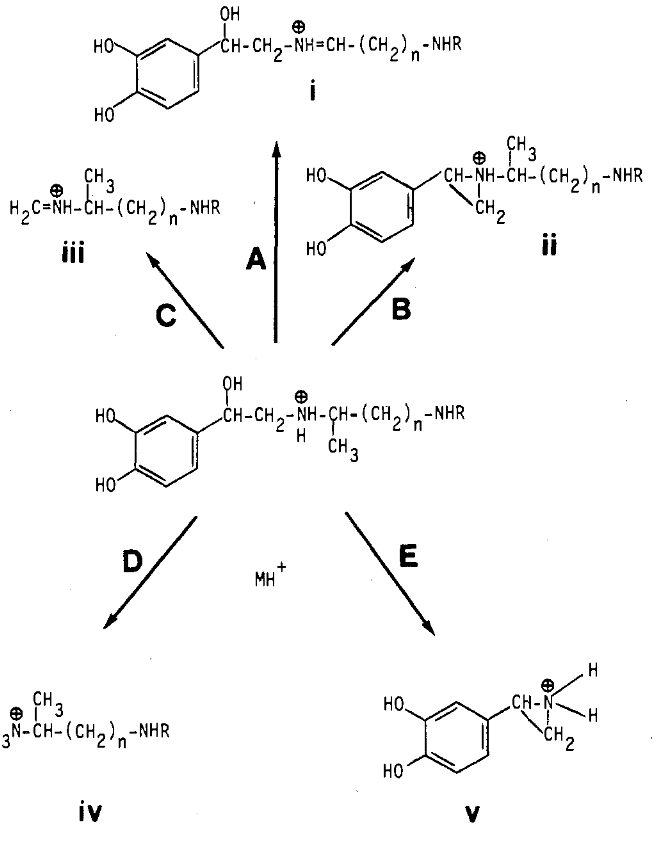


Figure 1. LSI mass spectrum of compound **25**.

Scheme V



(Table II). Loss of the isoalkyl methyl group as CH_3 (pathway A) and water (pathway B) lead to fragment ions i and ii, respectively. Cleavages of the side chain β (pathway c) and α (pathway d) to the norepinephrine nitrogen atom generate fragment ions iii and iv, respectively. Finally, all spectra displayed a fragment ion at m/e 152 (pathway E, structure v), which results from cleavage of the norepinephrine moiety. Ions ii and v could also arise from a protonated enamine derived from direct dehydration of the phenethylamine side chain. These mass spectral results are completely consistent with the structures assigned to these products.

The products **8–27** were prepared as mixtures of diastereomers; for this reason, melting points are not recorded. Partial resolution of the diastereomers of compounds **17** and **21** on HPLC was possible, however, as equal unseparated peaks.

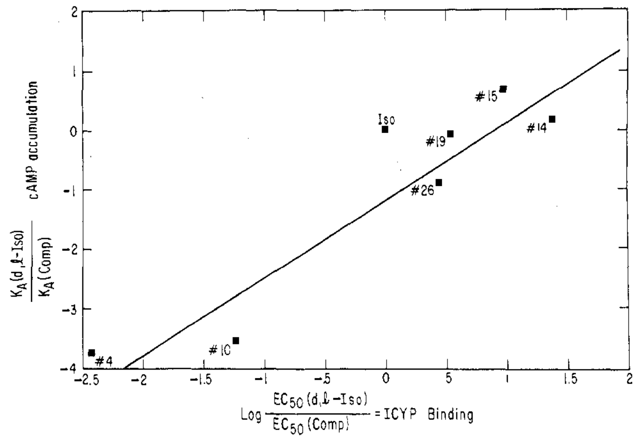


Figure 2. The log of the ratios of the EC_{50} of isoproterenol versus test compounds **10**, **14**, **15**, **19**, **26**, and **4** in ICYP binding competitive experiments and cyclic AMP accumulations in S49 cells.

Biological Results

The catecholamine derivatives were screened for biological activity by using the response (intracellular accumulation of cyclic AMP) of wild type S49 mouse lymphoma cells.[8] The response of these cells has been well characterized and shown to be dependent on an intact β-adrenergic receptor (predominantly β_2) that couples to adenylate cyclase. The activity of each compound was measured as a K_A (concentration of drug for half-maximal activity) in molarity units and an ϵ_{max} (maximum response or efficacy) in units of picomoles of cyclic AMP found in 10^7 cells. Each active compound was competitively and completely blocked by the β-adrenergic blocker propanolol (data not shown but details of the experiment are described in the footnote for Table I). The ratio of the K_A's for isoproterenol (tested simultaneously) and the test compound is considered an indicator of relative potency, and these ratios are listed in Table I. The ratios of test compound/isoproterenol were necessary to use because the compounds were tested over a 3-year period, and the potency of isoproterenol in our hands would vary over months of time. Therefore, we validated the rank potency ordering of the compounds by comparing the EC_{50} of a series of representative structures in competition with ^{125}[I]iodocyanopindolol (ICYP) for β receptors with their K_a for the activation of cyclic AMP (Figure 2).

These experiments were carried out simultaneously for a subgroup of the compounds that had been tested previously (Table I) for their ability to activate cAMP production. The compounds included **10**, **14**, **15**, **19**, **26**, **4**, and isoproterenol. Methods for ICYP binding have been described previously.[1e] The figure shows very close correlation of the log of the ratios of the EC_{50} of isoproterenol/EC_{50} of the tested compound for ICYP competition plotted against the log of the ratios of the compounds for their ability to stimulate production of cyclic AMP. The regression coefficient is 0.898, indicating a statistically significant correlation ($p < 0.01$). The EC_{50} of isoproterenol in the ICYP binding was $(9.8 \pm 0.2) \times 10^{-7}$ M (SEM). Its K_A for cyclic AMP production is $(1.2 \pm 0.2) \times 10^{-7}$ M (SEM). The EC_{50}'s of the experimental compounds in the ICYP binding experiments ranged from 4.9×10^{-8} (compound **14**) to 1.6×10^{-4} (compound **4**) and in the cAMP assay 8.7×10^{-9} (compound **15**) to 7.6×10^{-5} (compound **4**). The rank order of potency produced by this series of experiments for binding would be as follows: **14** > **15** > **19** > **26** ~ iso > **10** > **4**. The order for cyclic AMP would be as follows: **15** > **14** > iso > **19** > **26** > **10** > **4**. If we were to have used the data on ratios of cyclic AMP accumulation of isoproterenol to test compound accumulated over years of testing, it would match up reasonably well, being **15** > **19** > iso > **14** > **26** > **4** > **10** (Table I). There would have been minimal exchange in rank order potency of the test compounds on the basis of their ability to generate cyclic AMP. We conclude from these experiments that the rank order potency of the full spectrum of compounds in both this and the following paper in this issue (in which two additional compounds were tested for their ability to displace ICYP) is reliable.

The amine congeners **4** and **8–11**, which have a net charge of +2 at physiological pH, all had relatively low in vitro potencies (4–7 orders of magnitude less than that of isoproterenol). However, when the primary amine groups of these compounds were acylated by a variety of methods, so that the net charge on the molecule was restored to +1, the biological activity was generally increased. In the case of the epinephrine-like congener **4**, potency was enhanced significantly through acetylation (compound **6**) or especially through formation of the aromatic urea derivative **5**, which was, however, still approximately 2 orders of magnitude less potent than isoproterenol.

In the series of isoproterenol-like congeners **8–11** acylation led to more dramatic increases in potency. The (benzyloxycarbonyl)carbamate derivatives **12–15**, for example, in which the total length of the branched spacer group ($n + 1$) was varied from three carbons to six carbons, were all highly active. There was a marked dependence of potency on the length of the spacer, the most potent compound being the one that contained five methylenes (compound **15**), which was 40 times more potent than isoproterenol. The effect of the length of the spacer was also noted previously for carboxylic acid congeners and model amides.[1c] In this series of carbamate congener derivatives, however, contrary to some of our previous results,[1c] there was no decrease in potency when the side chain lacked an aromatic ring (compound **16** vs. compound **13**, Table I).

The *o*-methyl aromatic amide model compounds **21–24** showed virtually no biological activity. A similar lack of activity caused by ortho substitution of an aromatic ring in the side chain of isoprotrenol derivatives has been observed previously.[1c,17] The *p*-methyl amides **17–20**, however, showed a pronounced effect of chain length upon biological activity, much more so than in compounds in which the direction of the amide bond was reversed.[1c] The compound in which $n = 4$ (see Table I, compound **19**) was the most potent of the series as was the case in the reversed amides;[1c] it was roughly equipotent with isoproterenol—somewhat less potent than the analogue in which the amide bond is reversed.[1c] Surprisingly, the sulfonamide **25** was only slightly less active than isoprotrenol but 5 orders of magnitude more potent than the corresponding carboxamide **17**. The urea **26**, with roughly the same chain length as the amide **20**, exhibited activity 3 orders of magnitude less than that of isoproterenol. The dipeptide conjugate **27** was virtually inactive. However, when this dipeptide was employed previously in a peptide–drug conjugate, it also lowered the activity significantly relative to the model compound, although the in vivo activity of the conjugate was high.[1f]

Conclusions

The studies we have described further confirm our earlier observations[1c–e] that the biological activity of β-adrenergic agonists is dramatically dependent upon structural features of modifications of the *N*-isopropyl group of isoprotrenol. The extreme variations in potency, which are strongly dependent on both the length of the spacer group and also relatively minor structural modifications (e.g., *o*- vs. *p*-methyl) in the amine-substituent group, can be directly attributed to variations in binding affinity of these derivatives to the receptor.

We are currently extending our studies to the synthesis and biological activity of conjugates derived from these and other congeners of isoproterenol.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 180 spectrophotometer. High-resolution proton NMR spectra were taken on a Varian HR-360 spectrometer equipped with a Nicolet 1080 computer. Chemical shifts are reported as ppm downfield from $(CH_3)_4Si$. LSI mass spectra were taken on a Kratos MS50S mass spectrometer operating at a scan rate of 30 s/decade. The samples (ca. 50 μg) were dissolved in a mixture of glycerol and methanol (ca. 10:1). Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where elemental analyses are reported by symbols of elements, the results were within ±0.4% of the calculated value. Solvents were reagent grade except where indicated. *N*,*N*-Dimethylformamide (DMF) was distilled from polyphosphoric acid. Tetrahydrofuran (THF) and methylene chloride (CH_2Cl_2) were distilled from calcium hydride. Pyridine was distilled from barium oxide. The HPLC was performed with a Waters M-6000 pump and a Schoeffel GM-770 detector monitoring at 254 nm. Preparative TLC was conducted on Merck 2000-μm silica gel plates.

dl-Norepinephrine hydrochloride was purchased from Calbiochem, and the free base was prepared according to ref 1c. The compounds 4-oxopentanoic acid (levulinic acid) and 5-oxahexanoic acid were purchased from the Aldrich Chemical Co. The compounds 6-oxoheptanoic acid,[18] 7-oxooctanoic acid,[19] *N*-benzyl-3-chloropropylamine hydrochloride (**29**),[20] and the acetophenone **31**[21] were prepared by literature procedures. Although spectral data are reported only where deemed important, ^{1}H NMR, and in many cases IR, spectra were recorded for new numbered compounds and were judged to be consistent with the assigned structures. The pharmacological methods were the same as those used in ref 1c.

***N*-Benzyl-3-phthalimidopropylamine Hydrochloride (30).** The amine **29** (30.8 g, 0.14 mol) was dissolved in CH_2Cl_2 and shaken with 1 N NaOH. The aqueous layer was washed twice with CH_2Cl_2, and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was mixed with 52 g (0.28 mol) of freshly prepared potassium phthalimide in distilled DMF (175 mL). The solution was heated at 115 °C for 1 h. After the solution was cooled, 600 mL of H_2O was added and the mixture was acidified with 1 N HCl. The precipitate was filtered and washed with boiling H_2O. The filtrate was washed with CH_2Cl_2 to remove the last traces of phthalimide, saturated with NaCl, and extracted five times with *n*-BuOH. The combined *n*-BuOH extracts were evaporated, and the residue was recrystallized from EtOH, yielding **30** (20.7 g, 51%), mp 215–217 °C. Anal. ($C_{18}H_{19}N_2O_2Cl$) C, H, N.

3,4-Bis(benzyloxy)-α-[*N*-(3-phthalimidopropyl)-*N*-benzylamino]acetophenone (32). Compound **30** (12.8 g, 39 mmol) was dissolved in a slurry of CH_2Cl_2 and 0.2 N NaOH. The CH_2Cl_2 phase was separated, the aqueous phase was washed with CH_2Cl_2, and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to yield the free base of **30** (11.3 g, 98.5%). This amine (9.3 g, 31.6 mmol) was treated with compound **31** (6.4 g, 15.6 mmol) in dichloroethane (150 mL) at 80 °C for 4 h. The

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mixture was cooled to −15 °C, and the precipitated hydrobromide of **30** (5.1 g, 87%) was removed by filtration. The filtrate was evaporated, yielding **32** as a yellow oil. This compound was unstable and resisted attempts at purification by crystallization or chromatography and was therefore carried through to the next step. NMR ($CDCl_3$) δ 1.86 (m, 2 H, $CH_2CH_2CH_2$), 2.72 (t, 2 H, NCH_2CH_2), 3.69 (t, 2 H, phth-NCH_2CH_2), 3.82 and 3.75 (2 s, 2 H each, $C(=O)CH_2$ and $PhCH_2N$), 5.14 (s, 4 H, $PhCH_2O$), 6.86 (d, 1 H), 7.4 (m, 21 H).

1,2-Bis(benzyloxy)-4-[1-hydroxy-2-[*N*-(3-phthalimidopropyl)-*N*-benzylamino]ethyl]benzene (33). Crude ketone **32** (14.5 g, 23.2 mmol) was mixed with aluminum isopropoxide (70 g, 0.34 mol) in dry *i*-PrOH (300 mL). The mixture was distilled slowly with stirring, and *i*-PrOH was added when necessary to maintain a 300-mL volume. After 6 h, the mixture was cooled and saturated aqueous NaCl was added. The resulting syrup was extracted with Et_2O until the Et_2O extract was colorless. The organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on silica gel (5 × 30 cm) with a gradient of *i*-PrOH in CH_2Cl_2 (0–2%), yielding compound **33** as an oil (6.67 g, 45%). Anal. ($C_{40}H_{38}N_2O_5$) C, H, N.

1,2-Bis(benzyloxy)-4-[1-hydroxy-2-[*N*-(3-aminopropyl)-*N*-benzylamino]ethyl]benzene (34). To a solution of the alcohol **33** (6.4 g, 10.2 mmol) in EtOH (150 mL) was added hydrazine hydrate (3.07 g, 61.2 mmol). A voluminous precipitate was formed after refluxing for 2.0 h. The precipitate was decomposed by the addition of HOAc and heating for 5 min. The solution was then evaporated, redissolved in EtOH, filtered, and evaporated. The remaining oil was chromatographed on silica gel (5 × 30 cm), eluting first with $CHCl_3$/HOAc (95/5) and increasing the MeOH content to $CHCl_3$/HOAc/MeOH (75:5:20) to give pure compound **34** as an oil (4.2 g, 83%). Anal. ($C_{36}H_{44}N_2O_7$) H, N; C: calcd, 77.37; found, 76.78.

General Procedure for Acetylation of Compound 34. Intermediate **34** (1.0 mmol) was mixed with 1 mmol of acylating agent in 5–10 mL of distilled CH_2Cl_2. After stirring of the mixture for 10 min to 3 days, the solvent was evaporated, leaving the fairly pure acetylated derivatives **35**–**37**. The oily compounds were purified by preparative TLC on Merck 2000-μm silica gel plates, eluting with mixtures of EtOAc and MeOH, and were homogeneous by TLC (94–99% yield).

Compound **35**: NMR ($CDCl_3$) δ 1.66 (m, 2 H, $CH_2CH_2CH_2$), 2.66 (m, 2 H), 2.47 (m, 2 H), 3.22 (m, 2 H, $CH_2NHC{=}O$), 3.90 and 3.45 (dd, 2 H, OCH_2N), 4.66 (m, 1 H), 5.04 (s, 2 H), 5.08 (s, 2 H), 6.8 (m, 4 H), 7.3 (m, 20 H).

Compound **36**: NMR ($CDCl_3$) δ 1.63 (m, 2 H, $CH_2CH_2CH_2$), 1.82 (s, 3 H, $CH_3C{=}O$), 2.5 (m, 4 H), 3.20 (m, 2 H, $CH_2NHC{=}O$) 3.83 and 3.42 (dd, 2 H, OCH_2N), 4.61 (dd, 1 H), 5.12 (s, 4 H, OCH_2O), 5.84 (t, 1 H, $NHC{=}O$), 6.8 (m, 3 H), 7.3 (m, 15 H).

Compound **37**: NMR ($CDCl_3$) δ 1.66 (m, 2 H, $CH_2CH_2CH_2$), 1.96 (s, 3 H, $CH_3C{=}O$), 2.5 (m, 4 H), 3.3 (m, 2 H, $CH_2NHC{=}O$), 3.83 (d, 2 H, CH_2NHAc), 3.87 and 3.41 (dd, 2 H, $PhCH_2N$), 4.67 (dd, 1 H), 5.12 (s, 4 H, $PhCH_2O$), 6.8 (m, 4 H), 7.3 (m, 16 H).

Benzyl Carbamates 39–42. These compounds were prepared in 49–57% yield from the appropriate keto carboxylic acids. Satisfactory elemental analyses were obtained for C, H, and N.[13b,22] Only one representative preparation is given below.

Benzyl *N*-(3-Oxobutyl)carbamate (39). A solution of levulinic acid (3.4 g, 27.8 mmol), diphenylphosphoryl azide (7.65 g, 27.8 mmol), and distilled triethylamine (3.87 mL, 27.8 mmol) in 70 mL of benzene was stirred at 23 °C for 30 min and then refluxed for 15 min. Distilled benzyl alcohol (4.5 g, 42 mmol) was then added and the mixture refluxed for 17 h. The solvent was evaporated, and the residue was dissolved in 150 mL of EtOAc and washed with 50 mL of 5% aqueous citric acid and 30 mL each of H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The acid and neutral washes were extracted with 100 mL of EtOAc and the combined organic layers were dried ($MgSO_4$), filtered, and evaporated. The product was purified by flash chromatography[24] (EtOAc/hexanes, 4:6) to give compound **39** as a light yellow oil (3.55 g, 57%). Purification of a small quantity on a preparative TLC plate (Merck, 2000 μm; EtOAc/hexane, 4:6) gave an analytical sample. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

***N*-(5-Oxohexyl)-*N*′-(*p*-tolyl)urea (48).** To a solution of 6-oxoheptanoic acid (200 mg, 1.39 mmol), triethylamine (193 μL, 1.39 mmol), and 15 mL of benzene was added diphenylphosphoryl azide (0.299 mL, 1.39 mmol) under argon. The solution was refluxed for 30 min. *p*-Toluidine (74 mg, 0.7 mmol) was added, and after an additional 30-min reflux, the solution was cooled and added to EtOAc. The organic layer was washed with 0.5 N HCl, saturated aqueous $NaHCO_3$, and H_2O, dried ($MgSO_4$), filtered, and concentrated. The product was purified by preparative TLC (EtOAc/hexane, 55:45), giving **48** as a white solid, which was recrystallized from EtOAc/hexane (60 mg, 35%), mp 108–110 °C. Anal. ($C_{14}H_{20}N_2O_2{\cdot}0.35H_2O$) C, H, N.

Cyclohexyl *N*-(4-Oxopentyl)carbamate (49). To a solution of 5-oxohexanoic acid (200 mg, 1.54 mmol) and triethylamine (215 μL, 1.54 mmol) in 15 mL of benzene was added diphenylphosphoryl azide (0.331 mL, 1.54 mmol). The solution was refluxed for 30 min, cyclohexanol (120 μL, 1.15 mmol) was added, and the solution was refluxed an additional 15 h. The product was worked up in the same way as compound **48** (using EtOAc/hexane, 35:65, for the chromatography), yielding **49** as a light yellow oil (58 mg, 22%). Anal. ($C_{12}H_{21}NO_3$) C, H, N.

***N*$^{\alpha}$-Acetyl-*p*-[*N*′-(5-oxopentyl)ureido]-L-phenylalanylglycine Benzyl Ester (47).** A solution of 6-oxoheptanoic acid (189 mg, 1.3 mmol), triethylamine (0.181 mL, 1.3 mmol), and diphenylphosphoryl azide (0.28 mL, 1.3 mmol) was refluxed in 50 mL of THF for 1.5 h. The mixture was cooled to room temperature and to it was added a solution of the appropriate dipeptide[1b] (460 mg, 1.25 mmol) in 20 mL of THF/10 mL of DMF. After 15 min, 20 mL of H_2O was added, THF was evaporated, and the crude product was extracted into 100 mL of EtOAc. The organic layer was dried ($MgSO_4$), filtered, evaporated, and purified by preparative TLC ($CHCl_3$/MeOH/HOAc, 90:5:5), yielding compound **47** as a white solid (75 mg, 12%), which was recrystallized from MeOH/Et_2O/hexanes, mp 172–175 °C dec. Compound **47** was a single spot on TLC; $[\alpha]^{25}_{D}$ +20.2° (*c* 0.46, MeOH).

***N*$^{\alpha}$-Acetyl-*p*-[*N*′-(5-oxohexyl)ureido]-L-phenylalanylglycine Methylamide (50).** To a solution of compound **47** (50 mg, 0.098 mmol) in 30 mL of MeOH at 0 °C was added methylamine gas until the volume of the solution had approximately doubled. The mixture was allowed to warm to room temperature and stirred for 15 h, and the solvent was removed in vacuo. The residue was recrystallized from MeOH/Et_2O to give **50** as a white crystalline solid (27 mg, 63%): mp 161–163 °C; NMR (D_2O) δ 1.52 (m, 4 H), 1.96 (s, 3 H, $CH_3C{=}O$), 2.19 (s, 3 H, $CH_3C{=}O$), 2.57 (t, 3 H, $CH_2C{=}O$), 2.59 (s, 3 H, NCH_3), 3.02 (m, 2 H, NCH_2), 3.16 (t, 2 H, $PhCH_2$), 3.76 (dd, 2 H, Gly CH_2), 4.49 (t, 1 H, Phe CH), 7.20 (dd, 4 H); $[\alpha]^{25}_{D}$ 33.8° (*c* 0.9, H_2O). Anal. ($C_{21}H_{31}N_5O_3{\cdot}1.5MeOH$) C, H, N.

Keto Amides 43–46. These compounds were prepared as shown below in one example. Satisfactory elemental analyses for C, H, N were obtained for the mixtures. The overall yield of amide products in the reaction varied from 15% to 31%.

***N*-(3-Oxobutyl)-*p*-toluamide and *N*-(3-Oxobutyl)-*o*-toluamide (43).** A solution of levulinic acid (0.88 mL, 8.2 mmol), diphenylphoryl azide (1.86 mL, 8.2 mmol), and triethylamine (1.2 mL, 8.2 mmol) was stirred at 60 °C in 120 mL of toluene for 1 h. $AlCl_3$ (5 g) was then added, the solution was stirred at 50 °C for 2 h and allowed to cool to 23 °C, and H_2O was added carefully. the aqueous phase was separated, EtOAc was added to the organic phase, and the mixture was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The organic layer was dried ($MgSO_4$), filtered, and evaporated, and the crude product was purified by flash chromatography (EtOAc/hexanes, 4:6) to yield a mixture of 62% para and 38% ortho isomers that could not be separated easily chromatographically or by recrystallization (250 mg, 15%): NMR ($CDCl_3$) δ 2.24 (d, 3 H), 2.42 (d, 3 H), 2.82 nm, 2 H), 3.70 (m, 2 H), 6.41 (br s, 0.33 H), 6.81 (br s, 0.67 H), 7.25 (m, 2.66 H), 7.65 (d, 1.34 H, ortho H of *p*-$CH_3C_6H_4C{=}O$. Anal. ($C_{12}H_{15}NO_2{\cdot}0.6H_2O$) C, H, N.[13b,25]

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(22) The (2,4-dinitrophenyl)hydrazone of compound **39** was prepared, mp 107–109.5 °C (lit[23] mp 106–108 °C).

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***N*-(3-Oxobutyl)-*p*-toluenesulfonamide (51).** To a solution of *p*-toluenesulfonamide (1 g, 5.85 mmol) dissolved in 18 mL of $CHCl_3$ were added Al_2O_3 (1 g, neutral, Merck, activity grade I) and methyl vinyl ketone (0.64 mL, 7.0 mmol). The mixture was heated at 45 °C with stirring in a stoppered 50-mL round-bottom flask for 6 days. The solution was then filtered, and the Al_2O_3 was washed with 10 mL of EtOAc. Flash chromatography ($CHCl_3$/MeOH, 98:2) yielded **51** as a white solid (0.91 g, 65%): NMR ($CDCl_3$) δ 2.14 (s, 3 H, $CH_3C{=}O$), 2.44 (CH_3Ph), 2.72 (t, 2 H, $CH_2C{=}O$), 3.14 (q, 2 H, $NHCH_2$), 5.18 (t, 1 H, NH), 7.33 (d, 2 H,) 7.78 (d, 2 H). Anal. ($C_{11}H_{15}SNO_3$) C, H, N, S.

Method A. General Procedure for Deprotection of Compounds 34–37. The protected amine (1.0 mmol) was dissolved in dry CH_2Cl_2 and treated with an excess of 4 N HCl in dioxane. The solvent was evaporated under high vacuum. The residue was dissolved in 100 mL of MeOH/water mixture (80:20). The catalyst (10% Pd/C, 800 mg) was added and the mixture was hydrogenated under pressure (50 psi) for 24 h. The solution was filtered through diatomaceous earth, concentrated in vacuo and lyophilized. Compound **4** was recrystallized from EtOH/Et_2O. Compound **5** was purified by preparative TLC (*n*-BuOH/HOAc/H_2O, 4:1:5, upper phase). Compounds **6** and **7** were purified by semipreparative HPLC (Waters μ-Bondapak C-18 column, 0.1 N aqueous NaH_2PO_4).[14] In all cases, the purity was carefully checked by analytical HPLC (>99%) and 360-MHz proton NMR prior to biological testing.[13]

Compound **4**: NMR (D_2O) δ 1.88 (m, 2 H, $CH_2CH_2CH_2$), 2.9 (m, 6 H), 4.67 (m, 1 H, PhC*H*), 6.8 (m, 3 H), 7.2 (m, 5 H).

Compound **5**: NMR (D_2O) δ 1.82 (m, 2 H, $CH_2CH_2CH_2$), 3.1 (m, 6 H), 4.8 (m, 1 H, PhC*H*), 6.8 (m, 3 H), 7.2 (m, 5 H).

Compound **6**: NMR (D_2O) δ 1.77 (m, 2 H, $CH_2CH_2CH_2$), 1.84 (s, 3 H, $CH_3C{=}O$), 3.05 (m, 6 H), 4.77 (m, 1 H, PhC*H*), 6.75 (m, 3 H).

Compound **7**: NMR (D_2O) δ 1.69 (m, 2 H, $CH_2CH_2CH_2$), 1.82 (s, 3 H, $CH_3C{=}O$), 3.03 (d, 2 H, $CHOHCH_2N$), 2.86 and 3.09 (2 t, 2 H, $NCH_2CH_2CH_2N$), 3.63 (s, 2 H, Gly CH_2), 4.68 (t, 1 H, PhC*H*), 6.7 (m, 3 H).

Method B. Reductive Amination with Norepinephrine Using $NaCNBH_3$. A solution of the methyl ketone and *dl*-norepinephrine hydrochloride (Calbiochem, 1 equiv) in MeOH (2–5 mL) was mixed with anhydrous $NaCNBH_3$ (1–4 equiv). The solution was maintained in the pH 5–7 range by the addition of 10% HOAc in MeOH if necessary. After stirring at 50 °C for 1–3 days, a fivefold volume of 0.1 N HCl was added to decompose excess $NaCNBH_3$ (in the hood). The solution was then washed with $CHCl_3$, and the product was extracted into *n*-BuOH. The combined *n*-BuOH extracts were evaporated and the crude products were purified by reversed-phase HPLC with a 0.01 N HCl aqueous phase modified by MeOH if necessary. After purification, any MeOH present was evaporated, and the solution was lyophilized. Each derivative was then analyzed by HPLC again for purity (>99%). All spectral data were consistent with the assigned structures[13] and selected NMR data are given below. The average yield of compounds prepared by this procedure was 57%.

Compound **8**: NMR (D_2O) δ, 1.38 (d, 3 H, $CHCH_3$), 2.0 (m, 2 H), 3.06 (m, 2 H, CH_2NH_2), 3.22 (m, 2 H, $CHOHCH_2$), 3.42 (m, 1 H, C*H*NH), 6.9 (m, 3 H).

Compound **12**: NMR (D_2O) δ 1.20 (d, 3 H, $CHCH_3$), 1.8 (m, 2 H), 3.1 (m, 5 H), 5.14 (s, 2 H, $PhCH_2$), 6.9 (m, 3 H), 7.28 (s, 5 H).

Compound **17**: NMR (D_2O) δ 1.42 (d, 3 H, $CHCH_3$), 1.9 (m, 2 H), 2.40 (s, 3 H, $PhCH_3$), 3.4 (m, 5 H), 6.9 (m, 3 H), 7.30 (d, 2 H), 7.69 (d, 2 H).

Compound **21**: NMR (D_2O) δ 1.41 (dd, 3 H, $CHCH_3$), 1.9 (m, 2 H), 2.31 (2 d, 3 H, $PhCH_3$), 3.32 (m, 2 H, $CHOHCH_2$), 3.42 (t, 2 H, $CH_2NHC{=}O$).

Compound **27**: NMR (D_2O) δ 1.29 (d, 3 H, $CHCH_3$), 1.5 (m, 6 H), 1.94 (s, 3 H, $CH_3C{=}O$), 2.68 (s, 3 H, $NHCH_3$), 3.0 (dd, 2 H, $PhCH_2$), 3.20 (m, 2 H, $CHOHCH_2$), 3.32 (m, 1 H, NHC*H*CH_3), 3.56 (dd, 2 H, $CH_2CH_2NHC{=}O$), 3.85 (m, 2 H, Gly CH_2), 4.47 (t, 1 H, Phe C*H*), 6.9 (m, 3 H), 7.18 (br s, 4 H).

Method C. Reductive Amination with PtO_2. To a solution of the sulfonamide **51** (50 mg, 0.21 mmol) and *dl*-norepinephrine (0.21 mmol) in 1 mL of HOAc was added 5 mg of PtO_2. The solution was stirred under 1 atm of hydrogen for 40 h. The catalyst was removed by decantation and the HOAc solution was added to 5 mL of 0.1 N HCl. This solution was washed with $CHCl_3$, and the product was extracted into *n*-BuOH. The combined *n*-BuOH extracts were evaporated, and the product was purified on a Waters μ-Bondapak C-18 column (30% MeOH/70% 0.01 N HCl, 73% yield). The resultant compound **26** was homogeneous by HPLC (>99%)[13a] and TLC.

Method D. Removal of the Benzyloxycarbonyl Groups from Compounds 12 and 13. After the usual workup of the reaction between norepinephrine and compounds **39** and **40** (prior to HPLC purification), the resultant crude products were dissolved in HOAc and 10% Pd/C (10% by weight) was added. The mixture was stirred under 1 atm of hydrogen for 100 h, during which time TLC ($CHCl_3$/MeOH/HOAc, 70:15:15) showed that the benzyloxycarbonyl groups were being removed slowly. The solution was then decanted from the catalyst, added to 0.1 N HCl, and washed with *n*-BuOH. The air-sensitive products **8** and **9** were then purified on a Whatman ODS-3 C-18 HPLC column 0.01 N HCl)[13a] until homogeneity was achieved (>99% by HPLC).

Method E. Removal of the Benzyloxycarbonyl Groups from Compounds 14 and 15. After the workup of compounds **14** and **15** following reductive amination, the resultant crude products were dissolved in 31% HBr/HOAc and stirred for 30 min. The HBr was evaporated in the hood, Et_2O was added, and the liquid was decanted. The precipitates were dissolved in 0.1 N HCl and washed with *n*-BuOH. The products were purified on a Whatman ODS-3 C-18 column (0.01 N HCl). Compounds **14** and **15** were homogeneous by HPLC (>99%) and TLC.

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Registry No. (±)-4, 95483-63-9; (±)-4·2HCl, 95483-64-0; (±)-**5**, 95483-65-1; (±)-**5**·HCl, 95483-66-2; (±)-**6**, 95483-67-3; (±)-**6**·H_3PO_4, 95483-68-4; (±)-**7**, 95483-69-5; (±)-**7**·H_3PO_4, 95483-70-8; (±)-**8** (isomer 1), 95483-71-9; (±)-**8** (isomer 2), 95484-32-5; (±)-**8**·2HCl (isomer 1), 95483-72-0; (±)-**8**·2HCl (isomer 2), 95484-33-6; (±)-**9** (isomer 1), 95483-73-1; (±)-**9** (isomer 2), 95484-34-7; (±)-**9**·2HCl (isomer 1), 95483-74-2; (±)-**9**·2HCl (isomer 2), 95484-35-8; (±)-**10** (isomer 1), 95483-75-3; (±)-**10** (isomer 2), 95484-36-9; (±)-**10**·2HCl (isomer 1), 95483-76-4; (±)-**10**·2HCl (isomer 2), 95484-37-0; (±)-11 (isomer 1), 95483-77-5; (±)-11 (isomer 2), 95484-38-1; (±)-11 (isomer 1), 95483-78-6; (±)-11 (isomer 2), 95484-39-2; (±)-**12** (isomer 1), 95483-79-7; (±)-**12** (isomer 2), 95484-40-5; (±)-**12**·HCl (isomer 1), 95483-80-0; (±)-**12**·HCl (isomer 2), 95484-41-6; (±)-**13** (isomer 1), 95513-69-2; (±)-**13** (isomer 2), 95484-42-7; (±)-**13**·HCl (isomer 1), 95483-81-1; (±)-**13**·HCl (isomer 2), 95484-43-8; (±)-14 (isomer 1), 95483-82-2; (±)-14 (isomer 2), 95484-44-9; (±)-14·HCl (isomer 1), 95483-83-3; (±)-14·HCl (isomer 2), 95484-45-0; (±)-**15** (isomer 1), 95483-84-4; (±)-**15** (isomer 2), 95484-46-1; (±)-**15** (isomer 1), 95483-85-5; (±)-**15** (isomer 2), 95484-47-2; (±)-**16** (isomer 1), 95483-86-6; (±)-**16** (isomer 2), 95484-48-3; (±)-**16**·HCl (Isomer 1), 95483-87-7; (±)-**16**·HCl (isomer 2), 95484-49-4; (±)-**17** (isomer 1), 95483-88-8; (±)-**17** (isomer 2), 95484-50-7; (±)-**17**·HCl (isomer 1), 95483-89-9; (±)-**17**·HCl (isomer 2), 95484-51-8; (±)-**18** (isomer 1), 95483-90-2; (±)-**18** (isomer 2), 95484-52-9; (±)-**18**·HCl (isomer 1), 95483-91-3; (±)-**18**·HCl (isomer 2), 95484-53-0; (±)-**19**

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(25) *N*-(3-Oxobutyl)-*p*-toluamide was also prepared from *p*-toluoyl chloride and 3-oxobutylamine generated from compound **39** upon treatment with 31% HBr/HOAc and neutralization of the HBr salt with *N*-methylmorpholine. Anal. ($C_{13}H_{17}NO_2$) C, H, N (mp 79–81 °C).[26]

(26) Tsurutani, R.; Goodman, M., unpublished results.

(isomer 1), 95483-92-4; (±)-**19** (isomer 2), 95484-54-1; (±)-**19** (isomer 1), 95483-93-5; (±)-**19** (isomer 2), 95484-55-2; (±)-**20** (isomer 1), 95483-94-6; (±)-**20** (isomer 2), 95484-56-3; (±)-**20**·HCl (isomer 1), 95483-95-7; (±)-**20**·HCl (isomer 2), 95484-57-4; (±)-**21** (isomer 1), 95483-96-8; (±)-**21** (isomer 2), 95484-58-5; (±)-**21**·HCl (isomer 1), 95483-97-9; (±)-**21**·HCl (isomer 2), 95484-59-6; (±)-**22** (isomer 1), 95483-98-0; (±)-**22** (isomer 2), 95484-60-9; (±)-**22**·HCl (isomer 1), 95483-99-1; (±)-**22**·HCl (isomer 2), 95484-61-0; (±)-**23** (isomer 1), 95484-00-7; (±)-**23** (isomer 2), 95484-62-1; (±)-**23**·HCl (isomer 1), 95484-01-8; (±)-**23**·HCl (isomer 2), 95484-63-2; (±)-**24** (isomer 1), 95484-02-9; (±)-**24** (isomer 2), 95512-30-4; (±)-**24**·HCl (isomer 1), 95484-03-0; (±)-**24**-HCl (isomer 2), 95484-64-3; (±)-**25** (isomer 1), 95484-04-1; (±)-**25** (isomer 2), 95484-65-4; (±)-**25**·HCl (isomer 1), 95484-05-2; (±)-**25**·HCl (isomer 2), 95484-66-5; (±)-**26** (isomer 1), 95513-70-5; (±)-**26** (isomer 2), 95484-67-6; (±)-**26**·HCl (isomer 1), 95484-06-3; (±)-**26**·HCl (isomer 2), 95484-68-7; **27**, 95484-07-4; **27**·HCl, 95484-08-5; **29**, 42245-33-0; **30**, 95484-09-6; **30**·HCl, 95484-10-9; **31**, 27628-05-3; **32**, 95484-11-0; (±)-**33**, 95484-12-1; (±)-**34**, 95484-13-2; (±)-**35**, 95484-14-3; (±)-**36**, 95484-15-4; (±)-**37**, 95484-16-5; (±)-**38**, 586-17-4; **39**, 95484-17-6; **40**, 95484-18-7; **41**, 95484-19-8; **42**, 95484-20-1; **43** (*o*-methyl), 95484-21-2; **43** (*p*-methyl), 95484-22-3; **44** (*o*-methyl), 95484-23-4; **44** (*p*-methyl), 95484-24-5; **45** (*o*-methyl), 95484-25-6; **45** (*p*-methyl), 95484-26-7; **46** (*o*-methyl), 95484-27-8; **46** (*p*-methyl), 95484-28-9; **47**, 95513-71-6; **48**, 95484-29-0; **49**, 95484-30-3; **50**, 95484-31-4; **51**, 82125-95-9; $CH_3CONHCH_2CO_2C_6H_4NO_2$-*p*, 3304-61-8; $CH_3CO(CH_2)_3CO_2H$, 3128-06-1; $CH_3CO(CH_2)_4CO_2H$, 3128-07-2; $CH_3CO(CH_2)_5CO_2H$, 14112-98-2; PhNCO, 103-71-9; *p*-$NO_2C_6H_4OAc$, 830-03-5; $CH_3COCH_2CH_2CO_2H$, 123-76-2; *N*-(3-oxobutyl)-*p*-toluamide, 95484-22-3; 3-oxobutylamine, 23645-04-7; *dl*-norepinephrine hydrochloride, 55-27-6; potassium phthalimide, 1074-82-4; *p*-toluidine, 106-49-0; cyclohexanol, 108-93-0; methyl vinyl ketone, 78-94-4; *p*-toluoyl chloride, 874-60-2; L-Ac-Phe(NH_2)-Gly-OCH_2Ph, 88555-31-1.

Supplementary Material Available: The HPLC parameters and 360-MHz ^1H NMR data for compounds **4**–**27** (5 pages). Ordering information is given on any current masthead page.

Conjugates of Catecholamines. 6. Synthesis and β-Adrenergic Activity of *N*-(Hydroxyalkyl)catecholamine Derivatives[1]

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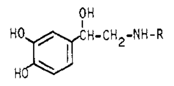
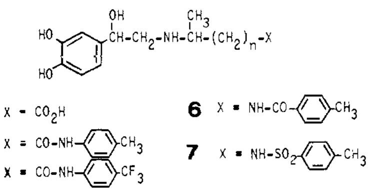
A new series of catecholamines has been prepared in which the *N*-alkyl substituent of *dl*-epinephrine or *dl*-isoproterenol has been extended by a methylene chain terminated by a hydroxyl group or derived functionality (e.g., carbamate or ester). These functionalized catecholamines (congeners) and model compounds were prepared with the goal of eventual attachment to polymeric carrier molecules. The β-adrenergic agonist activity of the derivatives was evaluated in vitro by measuring the intracellular accumulation of cyclic AMP in S49 mouse lymphoma cells and by the displacement of iodocyanopindolol (ICYP). A *n*-butylcarbamate derivative (compound **15**) was the most active compound in this series with a potency 190 times greater than *dl*-isoproterenol in the S49 assay. The biological results indicate that minor modifications in structure in the *N*-alkyl substituent of the catecholamine can influence the pharmacologic activity.

β-Adrenergic drugs such as epinephrine (**1**) and isoproterenol (**2**) have been the subject of extensive structure–activity studies.[2] As a result, virtually every part of the isoproterenol molecule has been modified in an attempt to obtain more selective or longer acting drugs. As part of our program to attach drugs covalently to polymeric carriers, we have prepared several series of functionalized catecholamines.[3] The most promising of these contain a functionalized *N*-alkyl substituent such as the carboxylic acid congeners **3**.[3d] Model derivatives such as compounds **4**–**7** have been synthesized in order to optimize the chemistry of linkage between the drug and carrier. Several of these model compounds have shown interesting pharmacological activities.[3e–f] For example, compound **5** ($n = 4$) has proven to be an extremely potent β-agonist when evaluated in both in vitro[3d,f] and in vivo[3e,f] test systems.



1 R = CH_3

2 R = $CH(CH_3)_2$



3 X = CO_2H

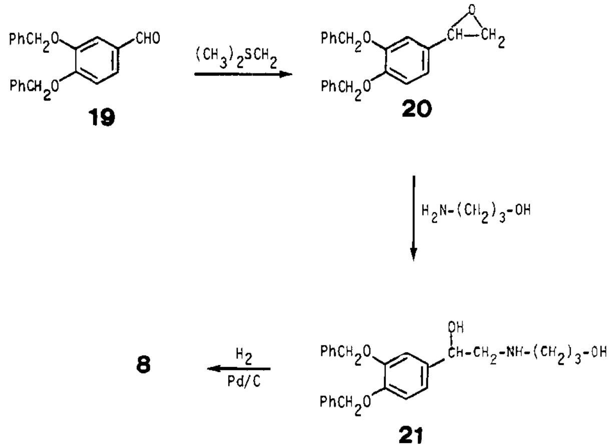
4

5

6

7

Scheme I



Here we describe the synthesis and evaluation of a series of *N*-(hydroxyalkyl)norepinephrine derivatives **8**–**18** (Table

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(1) For part 5 in this series, see: Reitz, A. B.; Sonveaux, E.; Rosenkranz, R. P.; Verlander, M. S.; Melmon, K. L.; Hoffman, B. B.; Akita, Y.; Castagnoli, N.; Goodman, M. *J. Med. Chem.*, preceding paper in this issue.

(2) For a review, see: Philips, D. *Handb. Exp. Pharm.* **1980**, *54/I*, 3–63.

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