(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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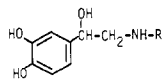
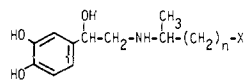
A new series of catecholamines has been prepared in which the *N*-alkyl substituent of *dl*-epinephrine or *dl*-isoproterenol has been extended by a methylene chain terminated by a hydroxyl group or derived functionality (e.g., carbamate or ester). These functionalized catecholamines (congeners) and 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.

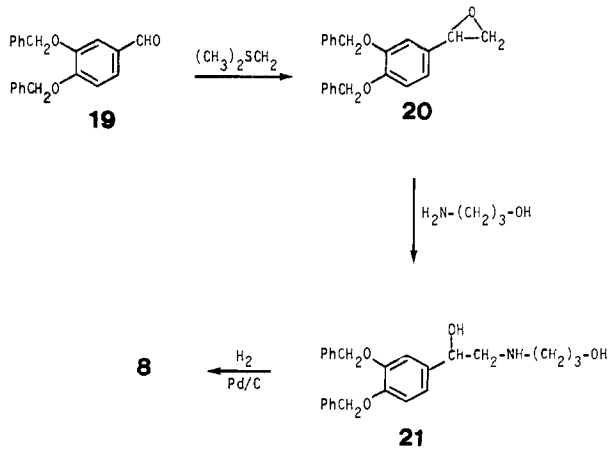
β-Adrenergic drugs such as epinephrine (**1**) and isoproterenol (**2**) have been the subject of extensive structure–activity studies.[2] As a result, virtually every part of the isoproterenol molecule has been modified in an attempt to obtain more selective or longer acting drugs. As part of our program 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 X = CO-NH-(2-naphthyl)-CH_3
5 X = CO-NH-(3-CF_3-phenyl)
6 X = NH-CO-(4-CH_3-phenyl)
7 X = $NH-SO_2$-(4-CH_3-phenyl)

Scheme I



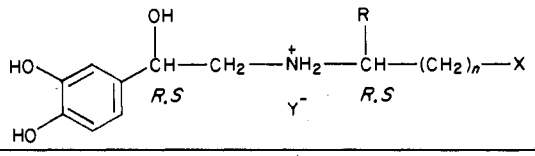
Here we describe the synthesis and evaluation of a series of *N*-(hydroxyalkyl)norepinephrine derivatives **8**–**18** (Table

[†] University of California, San Diego.
[‡] Stanford University Medical Center.
[§] University of California, San Francisco.

(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.

0022-2623/85/1828-0642$01.50/0

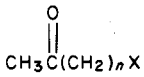
Table I. In Vitro Biological Activity of *N*-(Hydroxyalkyl)norepinephrine Derivatives



compd no.	R	*n*	X	rel potency	yield, %	method	formula
8	H	2	OH	5.8×10^{-3}	43	A	$C_{11}H_{17}NO_4 \cdot H_3PO_4$
9	CH_3	2	OH	1.2×10^{-2}	30	A	$C_{12}H_{19}NO_4 \cdot H_3PO_4$
10	CH_3	3	OH	1.3	32	A	$C_{13}H_{21}NO_4 \cdot H_3PO_4$
11	CH_3	4	OH	1.3×10^{-5}	10	A	$C_{14}H_{23}NO_4 \cdot H_3PO_4$
12	CH_3	3	$OCONH(C_6H_4)$-4-Me	9.2×10^{-2}	10	A	$C_{21}H_{28}N_2O_5 \cdot H_3PO_4$
13	CH_3	4	$OCONH(C_6H_4)$-4-Me	1.3	8	A	$C_{22}H_{30}N_2O_5 \cdot H_3PO_4$
14	CH_3	3	$OCONH(c\text{-}C_6H_{11})$	2.9×10^{1}	42	B	$C_{20}H_{32}N_2O_5 \cdot HCl$
15	CH_3	3	$OCONH(CH_2)_3CH_3$	1.9×10^{2}	19	A	$C_{18}H_{30}N_2O_5 \cdot H_3PO_4$
16	CH_3	3	$OCONHCO(C_6H_4)$-4-Me	7.3×10^{1}	75	B	$C_{22}H_{28}N_2O_6 \cdot HCl$
17	CH_3	3	$OCONHSO_2(C_6H_4)$-4-Me	4.0×10^{-7}	12	A	$C_{21}H_{28}N_2O_7S \cdot HCl$
18	CH_3	3	$OCO(C_6H_4)$-4-Me	6.3×10^{-3}	15	A	$C_{21}H_{27}NO_5 \cdot H_3PO_4$

[a] As determined by assessing the accumulation of cyclic AMP in SV-49 mouse lymphoma cells relative to dl-isoproterenol. The K_A values for isoproterenol and the test compounds were determined from the biological effects at 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 significantly ($p < 0.05$) between experiments. Displacement of ICYP was determined on compounds **15** and **17** only. The EC_{50} displacement in S49 cells was 3.2×10^{-8} and 3.6×10^{-6} M. These data correlated well with biological activity as was illustrated in Figure 2 of the preceding paper. Eight concentrations of propranolol were used in blocking experiments (ranging 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. Cell points were the mean of triplicate experiments whose coefficient of variability was less than 10%. [b] Purified, isolated yields.

Table II. Methyl Ketones

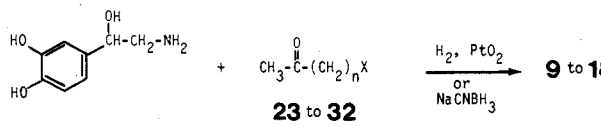


compd no.	*n*	X	mp, °C	recrystn solvent(s)	yield, %	formula[a]
26	3	$OCONH(C_6H_4)$-4-Me	113–114	EtOH	25	$C_{13}H_{17}NO_3$
27	4	$OCONH(C_6H_4)$-4-Me	94–95.5	CCl_4	31	$C_{14}H_{19}NO_3$
28	3	$OCONH(c\text{-}C_6H_{11})$	49–50.5	CCl_4	45	$C_{12}H_{21}NO_3$
29	3	$OCONH(CH_2)_3CH_3$	liq		28	$C_{10}H_{19}NO_5$
30	3	$OCONHSO_2(C_6H_4)$-4-Me	98.5–101.5	Et_2O	91	$C_{13}H_{17}NSO_5$
31	3	$OCONHCO(C_6H_4)$-4-Me	72–73.5	EtOAc/hexanes	25	$C_{14}H_{17}NO_4$[b]
32	3	$OCO(C_6H_4)$-4-Me	liq		49	$C_{13}H_{16}O_3$

[a] All compounds were analyzed for C, H, N. Analytical results were within ±0.4% of calculated values except where indicated. [b] Anal. Calcd for C: 63.86. Found: C, 64.27.

I).[4] In the hydroxyl congeners 8–11, the *N*-alkyl substituent of *dl*-epinephrine or *dl*-isproterenol has been replaced by an alkyl chain of varying length terminated by a hydroxyl group. This hydroxyl group has been derivatized in compounds **12–18** with either a carbamate or ester linkage which models the potential attachment of the congener to a polymeric carrier. Compounds 8–18 have been tested in an in vitro assay for β-adrenergic activity by measuring their ability to promote the accumulation of cyclic AMP in an S49 mouse lymphoma cell line.[1,5]

Scheme II



Congener **8** was prepared from 3,4-bis(benzyloxy)benzaldehyde (**19**) by the sequence of reactions shown in Scheme I. Aldehyde **19** was converted to epoxide **20** by treatment with dimethylsulfonium methylide.[3b,c,6,7] The epoxide was then opened with 3-aminopropanol to give a mixture of isomers from which benzylic alcohol **21** could be obtained chromatographically as a hemihydrate as established by elemental analysis. Catalytic hydrogenolysis afforded norepinephrine derivative **8** as a racemic pair of enantiomers.

Compounds **9–18** were prepared by reductive amination of the appropriate methyl hydroxyalkyl ketones or derivatives with *dl*-norepinephrine as the final step as shown in Scheme II.[1,3d] Commercially available *dl*-norepinephrine **22** was reacted with the methyl ketones **23–32** (Scheme II and Table II) by reductive amination with either Adam's catalyst (PtO_2)[8] or sodium cyanoboro-

(3) (a) Verlander, M. S.; Venter, J. C.; Goodman, M.; Kaplan, N. O.; Saks, B. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1009–1012. (b) Avery, M. A.; Verlander, M. S.; Goodman, M. *J. Org. Chem.* **1980**, *45*, 2750–2753; **1981**, *46*, 5459. (c) Reitz, A. B.; Avery, M. A.; Verlander, M. S.; Goodman, M. *J. Org. Chem.* **1981**, *46*, 4859–4863. (d) Jacobson, K. A.; Marr-Leisy, D.; Verlander, M. S.; Rosenkranz, R. P.; Melmon, K. L.; Goodman, M. *J. Med. Chem.* **1983**, *26*, 492–499. (e) Verlander, M. S.; Jacobson, K. A.; Rosenkranz, R. P.; Melmon, K. L.; Goodman, M. *Biopolymers* **1983**, *22*, 531–545. (f) 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.

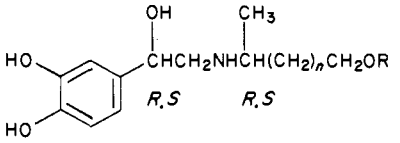
(4) Compound **8** has been prepared previously via a different route. Langecker, H.; Friebel, H. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **1955**, *226*, 493–504.

(5) (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.

(6) Sohda, S.; Fujimoto, M.; Tamegai, T.; Hirose, N. *J. Med. Chem.* **1979**, *22*, 279–286.

(7) Corey, E.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353–1363.

Table III. Protonated Molecular and Fragment Ions of *N*-(Hydroxyalkyl)catecholamine Derivatives Observed under LSI Mass Spectral Conditions



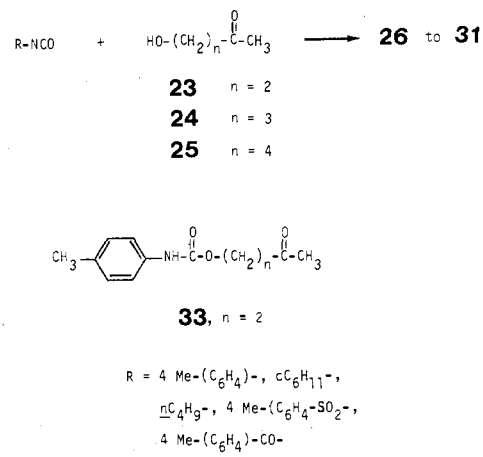
compd no.	*n*	R	MH^+	I	II	III	IV
11	1	H	270	254	252	130	118
13	3	$CONH(C_6H_4)$-4-Me	403	387	385	263	251
14	2	$CONH(c\text{-}C_6H_{11})$	381	365	363	241	229
15	2	$CONH(CH_2)_3CH_3$	355	339	337	215	203
16	2	$CONHCO(C_6H_4)$-4-Me	417			277	265
17	2	$CONHSO_2(C_6H_4)$-4-Me	453	437	435	313	301

hydride.[9] The hydrochloride salt of *dl*-norepinephrine was used with sodium cyanoborohydride, whereas the free base of *dl*-norepinephrine was employed with PtO_2. The yields reported in Table I are not optimized and are often the result of only a single experiment; however, the moderate to high yields associated with the use of sodium cyanoborohydride (method B) relative to the low yields obtained with PtO_2 (method A) indicate that the former is the preferred reagent for this reaction. Melting points for the final products are not reported since they are diastereomeric mixtures that melted over broad ranges with decomposition. That compounds **9–18** are indeed roughly equal mixtures of diastereomers is shown by the 360-MHz ^1H NMR spectra which shows the CH_3 protons as a doublet of doublets clearly resolved in some cases and by analytical HPLC in which several of the compounds partially separated into two overlapping, equal peaks.

Hydroxy ketones **23–25** were either commercially available or were prepared according to literature procedures.[10,11] Alcohols **23–25** were allowed to react with the appropriate isocyanates to yield methyl ketones **26–31** (see Scheme III). Keto ester **32** was prepared from reaction of keto alcohol **24** and *p*-tolyl chloride. Compounds **26–31** were fully characterized by physical and spectroscopic means and are listed in Table II. Compound **33** was also prepared, although it was unstable to silica gel chromatography.[12] Ketone **33** was also unstable to conditions of reductive amination, failing to give any of the expected catecholamine product.[13]

Final products **8–18** were first treated with an extractive workup as a preliminary purification step. They were then subjected to chromatography, either using reverse-phase semipreparative high-performance liquid chromatography (HPLC)[14] or flash chromatography.[15] The HPLC purification was essentially performed as described elsewhere[3d]

Scheme III

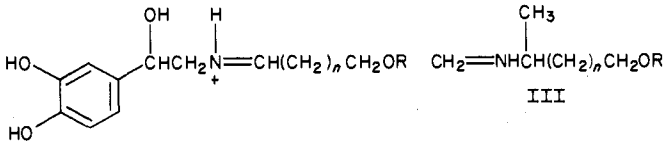


except that occasionally the phosphate buffer was replaced by a dilute HCl solution (0–50% MeOH in 0.01 N HCl, pH >2). The latter system offered the advantage that the final compounds were unambiguously the hydrochloric salts. With use of the phosphate buffer, the products were assumed to be the phosphate salts, a hypothesis supported by elemental analysis.[3d] Iterative purification by HPLC provided sufficient quantities of products (≥10 mg) for preliminary in vitro screening. Larger quantities (ca. 1 g) were prepared when required and purified by flash chromatography. Compounds **9–11** exhibited partial resolution of the diastereomeric pairs of enantiomers on analytical HPLC (vide supra). All of the final products were shown to be >99% pure by analytical HPLC, or a further purification step was undertaken. The 360-MHz ^1H NMR spectra fully substantiated the proposed structures.[16] Elemental analysis of compound **14**, prepared on large scale, completely agreed with the calculated values.

Several of the congeners were examined by liquid secondary ion mass spectrometry (LSIMS).[17] The spectra obtained with these compounds were very similar to those reported in the preceding paper.[1] In all instances an intense protonated molecular ion was observed as well as the fragment ion at *m/e* 152 (structure V, Table III) which is due to the cleavage of the norepinephrine moiety. All molecules except compound **16** displayed fragment ions corresponding to loss of CH_4 (structure I) and H_2O (structure II). Additionally, ions corresponding to the

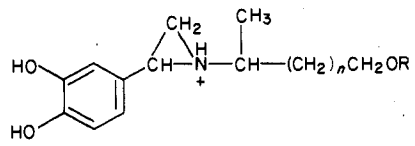
(8) Emerson, W. *Org. React.* **1948**, *4*, 174–255.
(9) (a) Borch, R.; Bernstein, M.; Durst, H. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904. (b) Stout, D.; Gorczynski, R. *J. Med. Chem.* **1982**, *25*, 326–328.
(10) White, T.; Howard, R. *J. Chem. Soc.* **1943**, 25–31.
(11) Perkin, W. H. *J. Chem. Soc.* **1887**, 702–748.
(12) Reitz, A.; Verlander, M.; Goodman, M. *Tetrahedron Lett.* **1982**, *23*, 751–752.
(13) The only catecholamine product from the reaction of **33** with norepinephrine (**22**) and $NaCNBH_3$ was *N*-2-butylnorepinephrine. This presumably arose by cleavage of **33** to methyl vinyl ketone, which then underwent reduction of the C–C double bond and reductive amination with **22**.
(14) (a) Molnar, I.; Horvath, C. *Clin. Chem.* **1976**, *22*, 1497–1502. (b) Scratchley, G. A.; Masoud, A. N.; Stoho, S. J.; Wingard, D. W. *Chromatographia* **1979**, *17*, 279–309. (c) Krstulovic, A. M. *Adv. Chromatogr.* **1979**, *17*, 279–309.
(15) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(16) (a) Supplementary material details the conditions for the HPLC analysis and gives complete 360-MHz ^1H NMR data for compounds **8–18**. (b) Reitz, A. B. Ph.D. Thesis, University of California, San Diego, 1982.
(17) Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029–2034.

iminium fragments (structure III) and ammonium fragments (structure IV) were present in all spectra. Structures II and V could also be viewed as protonated enamine species arising from dehydration of the phenethylamine side chain.

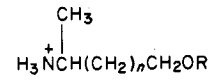


I

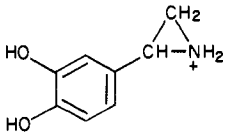
III



II

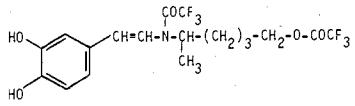


IV

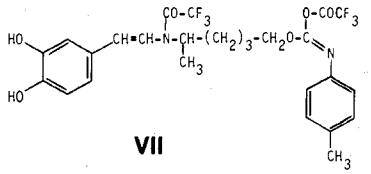


V

Congeners 11 and 13 were submitted to high-resolution electron-ionization mass spectrometry. In order to obtain adequate volatility for electron-ionization analysis, the compounds first were derivatized with trifluoroacetyl chloride. Since these derivatives subsequently were dissolved in methanol prior to application to the direct insertion probe, the trifluoroacetyl groups attached to the catechol oxygen atoms underwent selective solvolysis. An abundant ion at nominal mass 443 appeared in the electron-ionization spectrum of compound 11. The likely structure for this species is the fragment ion VI, which has an empirical formula of $C_{18}H_{19}F_6NO_5$ and corresponds to the parent minus CF_3COOH. The calculated exact mass for this fragment is 443.115 75 while the found exact mass was 443.116 040. In the case of compound 13, an abundant ion was observed at nominal mass 576 ($C_{26}H_{26}F_6N_2O_6$, structure VII is tentatively proposed; trifluoroacetylation could also occur on the carbamate nitrogen). The calculated exact mass for this species is 576.169 507; the found exact mass was 576.169 504.



VI



VII

Results and Discussion

The potential β-adrenergic activities of the *N*-(hydroxyalkyl)norepinephrine derivatives 8–18 were evaluated in vitro by measuring their ability to stimulate the intracellular accumulation of cyclic AMP in S49 mouse lymphoma cells.[5] Selected compounds were also tested for their capacity to displace [^{125}I]iodocyanopindolol, such results being consistent with the cyclic AMP stimulation data (data not shown). Each compound was tested concurrently with the prototypic β-agonist *dl*-isoproterenol, and this testing procedure has been validated as described in the preceding paper.[1] The ratio of K_A (association constant) for isoproterenol to the K_A for each compound gave a measure of relative potency for compounds 8–18. The biological activities of the final products as determined in this assay are listed in Table I. The activity of each compound was competitively and completely blocked by propranolol, indicating that a drug–receptor interaction at the β-receptor was responsible for the observed accumulation of cyclic AMP.

The *N*-hydroxyalkyl congeners 8–11 showed a dependence of biological activity upon the length of the *N*-alkyl group. Compound 10, the isoproterenol congener containing a branched, five-carbon chain, was the most active compound, with a potency approximately equal to that of isoproterenol. Compound 8, in which there is no methyl group α to the nitrogen (i.e., an epinephrine-related congener), showed the expected decrease in β-activity relative to compound 9.[18] The hydroxyalkyl compound in which $n = 4$ (11) exhibited unexpectedly low activity. However, when the hydroxyl groups of compound 10 or 11 were converted to carbamate derivatives (compounds 12–17), the activity was generally very high.

A variety of different carbamates were prepared and evaluated. Once again, there was a marked dependence of activity on the length of the *N*-alkyl chain. Compound 13, for example, was approximately equipotent to isoproterenol but about 15 times more potent than the closely analogous compound 12. These results are in agreement with the marked dependence of activity on chain length in carboxylic acid congeners and derived amides.[3d]

Both of the alkylcarbamates 14 and 15 were highly active. The most active compound prepared was the *n*-butylcarbamate 15, which was 190 times as potent as isoproterenol (2). The high activity of these alkyl carbamates vs. the aromatic derivatives 12 and 13 is somewhat surprising in view of the opposite effect observed for previously prepared amides of carboxylic acid congeners.[3d] The acylcarbamate 16 was roughly equipotent to isproterenol (2). Surprisingly, however, the sulfonylcarbamate 17 was virtually inactive as a β agonist. The ester 18 had an activity that was approximately 2 orders of magnitude lower than that of isoproterenol (2). Although it is difficult to generalize on the basis of a relatively small number of compounds, it is clear that several of the carbamate derivatives possess pronounced β-adrenergic activity (especially compound 15) and that the activity of the series 9–11 is chain-length dependent.

A binding study performed by Insel and co-workers examined the competitive displacement of [^{125}I]iodohydroxybenzylpindolol by compound 14.[19] These results indicated that the activity of compound 14 could be fully explained on the basis of the affinity of the compound for the β receptor, confirming our propranolol blocking results (vide supra).

Conclusions

Our study indicates that modifications of the *N*-alkyl substituents of epinephrine (1) or isoproterenol (2) with hydroxyalkyl functionalities or derivatives such as carbamates generally results in retention, or even a substantial enhancement, of potency in an in vitro assay for β-adrenergic activity. The highly active compounds presented

(18) Triggle, D. J. "Burger's Medicinal Chemistry"; Wiley: New York, 1980; Part III, pp 225–284.

(19) (a) Insel, P.; Stoolman, L. M. *Mol. Pharmacol.* **1978**, *14*, 549–561. (b) Insel, P.; Mahan, M.; Garst, A., unpublished results.

here (e.g., **14** and **15**), when evaluated in the context of previously prepared analogues,[2,3d-f,18] further establish the view[18] that a requirement for high β-adrenergic activity in the *N*-alkyl region of the catecholamine molecule is a combination of nonpolar (hydrophobic, steric) and ionic interactions (possibly hydrogen bonding). The extreme sensitivity of the receptor binding of the catecholamines to structural modifications is a promising indication that alteration of the side chain may provide more useful therapeutic agents. We are currently investigating further the pharmacological activity (e.g., selectivity at β_1 or β_2 receptors) of this new class of compounds.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are reported by symbols of elements, the results were within ±0.4% of the calculated value. Proton NMR spectra were recorded on a Varian HR-360 spectrometer in the Fourier-transform mode unless otherwise indicated. IR spectra were recorded on a Perkin-Elmer spectrophotometer. Preparative TLC was carried out on Merck 2000 μm silica gel plates.

LSI mass spectra were taken on a Kratos MS 50S mass spectrometer equipped with a Cs^+ gun and 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). High-resolution electron-minimization mass spectra were taken on an HEI MS 902S instrument using the peak matching technique with PFK ions of known composition serving as reference masses. The atom list monitored was ^{12}C, ^{13}C, H, N, O, S, and F. The samples (50 μg) were heated in trifluoroacetyl chloride (2 μL) at 60 °C for 20 min. Prior to analysis the solvent was removed under a stream of N_2 and the residue, in methanol, was applied to the direct insertion probe.

The compound 4-hydroxy-2-butanone (**23**) was prepared by the condensation of formaldehyde with acetone,[10] and 6-hydroxy-2-hexanone (**25**) was prepared by the method of Perkin.[11] *p*-Toluoyl isocyanate was prepared from *p*-toluamide.[20] Cyclohexyl isocyanate, *n*-butyl isocyanate, *p*-tolyl isocyanate, *p*-tosyl isocyanate, and 5-hydroxy-2-pentanone (**23**) were purchased from the Aldrich Chemical Co. *dl*-Norepinephrine hydrochloride was purchased from Calbiochem-Behring Corp., and *dl*-norepinephrine was prepared as described in ref 3d.

Although spectral data are reported only where considered important, NMR and, in many cases, IR spectra were recorded for new numbered compounds and were consistent with the designated structures. The synthesis of the methyl ketones **26–32** are exemplified by several examples.

1,2-Bis(benzyloxy)-4-[1-hydroxy-2-[(3-hydroxypropyl)-amino]ethyl]benzene Hydrochloride (21). The published procedure[3b,7] for the use of dimethylsulfonium methylide was used exactly with the following quantities: NaH (50% oil dispersion, 5.4 g, 0.11 mol), trimethylsulfonium iodide (23 g, 0.11 mol) in 90 mL of distilled Me_2SO, and the aldehyde **19** (30 g, 0.085 mol) in 180 mL of THF. The product **20** was a thick oil, produced in nearly quantitative yield from **19**, that decomposed on silica gel. To a solution of compound **20** (10 g, 30 mmol) in 125 mL of EtOH was added 3-hydroxypropylamine (20 mL). This mixture was refluxed for 72 h, after which time the EtOH was evaporated and the remaining oil was added to CH_2Cl_2. This solution was washed with water and 0.01 N HCl. The organic layer was separated, dried ($MgSO_4$), filtered, and evaporated. The crude product was chromatographed on silica gel (CH_2Cl_2/MeOH/HOAc, 90:8:2), collecting the slower moving spot on TLC.[21] The product was recrystallized from CH_2Cl_2/Et_2O to give **21** as an off-white solid, mp 92–95 °C (2.75 g, 26% yield). Anal. ($C_{25}H_{30}NO_4Cl\cdot0.5H_2O$) C, H, N.

***N*-(3-Hydroxypropyl)norepinephrine (8).** A solution of compound **21** (130 mg, 0.28 mmol) in 50 mL of HOAc containing 10% Pd/C (approximately 40 mg) was stirred for 15 h under 1 atm of hydrogen. The mixture was then filtered through diatomaceous earth under nitrogen, lyophilized, and then relyophilized from 0.1 N HCl. The crude product was purified on a Whatman Magnum ODS-3 HPLC column (0.1 N NaH_2PO_4) to give **8** as a white solid (43%) which was homogenous by TLC. NMR was consistent with the assigned structure.

4-Oxopentyl *N*-(*p*-Tolyl)carbamate (26). A solution of *p*-tolyl isocyanate (0.93 mL, 7.4 mmol) and distilled 5-hydroxy-2-pentanone (**24**: 0.75 mL, 7.5 mmol) was heated at 40 °C for 30 min. The resulting precipitate was recrystallized from EtOH and further purified by preparative TLC ($CHCl_3$/MeOH, 92:8) to yield **26** as a white solid (50 mg, 25%), mp 113–114 °C. NMR and IR were consistent with the assigned structure. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

4-Oxopentyl *N*-Cyclohexylcarbamate (28). To a mixture of 5-hydroxy-2-pentanone (**24**; 5 mL, 49.4 mmol) and cyclohexyl isocyanate (6.17 g, 60 mmol) in 20 mL of CH_2Cl_2 under nitrogen was added 2 drops of dibutyltin dilaurate. After 2 h the solvent was evaporated and the product was recrystallized from CCl_4, yielding **28** as a white solid (4.5 g, 45%), mp 49–50.5 °C. NMR and IR were consistent with the assigned structure. Anal. ($C_{12}H_{21}NO_3$) C, H, N.

4-Oxopentyl *N*-(*p*-Tolylsulfonyl)carbamate (30). To a solution of distilled 5-hydroxy-2-pentanone (1.5 mL, 14.7 mmol) in 2.5 mL of distilled chlorobenzene at −15 °C under nitrogen was added *p*-tolylsulfonyl isocyanate (2.9 mL, 14.7 mmol). The solution was allowed to warm to 23 °C and 5 mL of Et_2O was added. Crystallization yielded **30** as a white solid (4.0 g, 91%), mp 98.5–101.5 °C. NMR was consistent with the assigned structure. Anal. ($C_{13}H_{17}NSO_5$) C, H, N.

4-Oxopentyl *p*-Toluate (32). To a solution of *p*-toluoyl chloride (400 mg, 2.6 mmol) and 5-hydroxy-2-pentanone (**24**; 0.79 mL, 7.8 mmol) in 5 mL of CH_2Cl_2 was added distilled pyridine (0.533 mL, 6.5 mmol) at 0 °C. The mixture was allowed to warm to 23 °C and stirred for 30 min. The precipitate was filtered, and the filtrate washed with saturated aqueous NaCl and saturated aqueous $NaHCO_3$, evaporated, and chromatographed over silica gel (EtOAc/hexanes, 33:67). The resultant product **32** was a clear oil (280 mg, 49% yield). NMR and IR were consistent with the assigned structure. Anal. ($C_{13}H_{16}O_3$) C, H, O.

Reductive Amination of Methyl Ketones with *dl*-Norepinephrine. Representative examples are given below. Where HPLC purification was employed, the system used was a Waters M-6000 pump with a Schoeffel GM 770 detector set at 254 nm. The reverse-phase columns (Waters μBondapak C_{18} or Whatman Magnum ODS-3) were run with a 0.1 M NaH_2PO_4 solution modified with 0–50% MeOH; up to 2 mg could be purified with each injection. The product peak was collected, the MeOH evaporated, and the water lyophilized, and the NaH_2PO_4 was removed by dissolving the catecholamine in MeOH or EtOH. Compounds **16** and **17** were purified with 30% MeOH/70% 0.01 N HCl as the mobile phase (pH >2.0). After purification, analytical HPLC showed that the derivatives were homogeneous (>99%), or a further purification was performed.

Method A. *N*-(5-Hydroxy-2-pentyl)norepinephrine (10). To a solution of 5-hydroxy-2-pentanone (**24**; 122 mg, 1.2 mmol) and *dl*-norepinephrine (**22**; 116 mg, 1.2 mmol) in 1 mL of HOAc was added 15 mg of PtO_2. The solution was stirred under 1 atm of H_2 for 10 h. The solvent was decanted from the catalyst and added to an approximately fivefold volume of 0.1 N HCl. This solution was washed two times with 2 equal volumes of $CHCl_3$ to remove unreacted ketone and extracted three times into 3 equal volumes of *n*-BuOH. The combined *n*-BuOH extracts were evaporated, and the derivative was purified two times by reversed-phase HPLC,[16a,3d] yielding **10** as an amorphous solid. Only about 10 mg of product was purified rigorously in this manner; extrapolation to the total amount of crude product indicated a 32% purified reaction yield. The product was homogeneous by TLC and HPLC: NMR (D_2O) δ 6.99 (m, 2 H), 6.80 (d, 1 H), 3.59 (m, 2 H, CH_2OH), 3.32 (m, 1 H, NH_2CH), 3.22 (m, 2 H, NH_2CH_2),

(20) Speziale, A.; Smith, L. *J. Org. Chem.* **1962**, *27*, 3742–3743.
(21) We have found that where there are isomers arising from different positions of epoxide opening in related compounds that the slower moving spot is the desired benzyl alcohol and the faster moving spot is the isomeric benzylamine.[22]
(22) Goodman, M.; Verlander, M. S.; Melmon, K. L.; Jacobson, K. A.; Reitz, A. B.; Taulane, J. P.; Avery, M. A.; Kaplan, N. O. *Eur. Polym. J.* **1983**, *19*, 997–1004.

1.6 (m, 4 H), 1.28 (d, 3 H, CH_3). Benzylic CH was obscured under HOD peak.

Method B. *N*-[5-[[(Cyclohexylamino)carbonyl]oxy]-2-pentyl]norepinephrine Hydrochloride (14). A solution of compound **28** (1 g, 4.4 mmol), *dl*-norepinephrine hydrochloride (903 mg, 4.4 mmol), and $NaCNBH_3$ (416 mg, 6.6 mmol) in 35 mL of MeOH was adjusted to pH 6.0 by the addition of HOAc. The solution was then stirred for 15 h at 40 °C and then at room temperature for 36 h. Excess $NaCNBH_3$ was then destroyed by the addition of 50 mL of 0.1 N HCl; the HCN was removed under aspirator vacuum in the hood. The solution was washed three times with 50 mL of $CHCl_3$, and the product was extracted into 100 mL of *n*-BuOH. The *n*-BuOH layer was washed three times with 50 mL of H_2O and then evaporated. The product was purified by flash chromatography[16] using a gradient of 86:9:5 to 79:16:5 of $CHCl_3$/MeOH/HOAc. The appropriate fractions were evaporated, added to 100 mL of 0.1 N HCl, washed with $CHCl_3$ (3 × 50 mL), extracted into 100 mL of *n*-BuOH, and evaporated. The product was dissolved in H_2O and lyophilized to an amorphous white solid which was homogeneous by HPLC and TLC: NMR (D_2O) δ 6.99 (m, 2 H), 6.91 (d, 2 H), 4.14 (m, 2 H, CH_2O), 3.46 (m, 4 H), 1.7 (m, 14 H), 1.41 (d, 3 H, CH_3). Anal. ($C_{20}H_{33}N_2O_5Cl$) C, H, N, O.

SV-49 Mouse Lymphoma Cell Assay for Cyclic AMP. The method used was essentially that described in ref 3d and is summarized here. The cells were suspended in DME (13.3 g/L) and 20 mM Hepes (pH 7.4) with 0.1% BSA at a concentration of (2–2.5 × 10^6)/mL. They were then incubated for 10 min at 37 °C and added to solutions with or without test compounds for 6 min more. The solutions were cooled to 0 °C and centrifuged. The cell pellets were resuspended and boiled and cyclic AMP levels were determined by the method of Gilman.[5b] For each compound, a K_A (association constant in molarity units) and an E_{max} (maximal activity) was determined. Each K_A was the average of at least three determinations measured in triplicate. The relative activity is conveniently expressed as the ratio of K_A for isoproterenol (determined at the same time) to the K_A for the test compound. This ratio showed no significant variation ($p < 0.05$). The E_{max} for compounds 8–18 were roughly the same as for isoproterenol and are not reported here.

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Registry No. (±)-**8**, 95482-86-3; (±)-**8**·H_3PO_4, 95482-87-4; (±)-**9** (isomer 1), 95482-88-5; (±)-**9** (isomer 2), 95483-18-4; (±)-**9**·H_3PO_4 (isomer 1), 95482-89-6; (±)-**9**·H_3PO_4 (isomer 2), 95483-19-5; (±)-**10** (isomer 1), 95482-90-9; (±)-**10** (isomer 2), 95483-20-8; (±)-**10**·H_3PO_4 (isomer 1), 95482-91-0; (±)-**10**·H_3PO_4 (isomer 2), 95483-21-9; (±)-**11** (isomer 1), 95482-92-1; (±)-**11** (isomer 2), 95483-22-0; (±)-**11**·H_3PO_4 (isomer 1), 95482-93-2; (±)-**11**·H_3PO_4 (isomer 2), 95483-23-1; (±)-**12** (isomer 1), 95482-94-3; (±)-**12** (isomer 2), 95483-24-2; (±)-**12**·H_3PO_4 (isomer 1), 95482-95-4; (±)-**12**·H_3PO_4 (isomer 2), 95483-25-3; (±)-**13** (isomer 1), 95482-96-5; (±)-**13** (isomer 2), 95483-26-4; (±)-**13**·H_3PO_4 (isomer 1), 95482-97-6; (±)-**13**·H_3PO_4 (isomer 2), 95483-27-5; (±)-**14** (isomer 1), 95482-98-7; (±)-**14** (isomer 2), 95483-28-6; (±)-**14**·HCl (isomer 1), 95482-99-8; (±)-**14**·HCl (isomer 2), 95483-29-7; (±)-**15** (isomer 1), 95483-00-4; (±)-**15** (isomer 2), 95483-30-0; (±)-**15**·H_3PO_4 (isomer 1), 95483-01-5; (±)-**15**·H_3PO_4 (isomer 2), 95483-31-1; (±)-**16** (isomer 1), 95483-02-6; (±)-**16** (isomer 2), 95483-32-2; (±)-**16**·HCl (isomer 1), 95483-03-7; (±)-**16**·HCl (isomer 2), 95483-33-3; (±)-**17** (isomer 1), 95512-29-1; (±)-**17** (isomer 2), 95483-34-4; (±)-**17**·HCl (isomer 1), 95483-04-8; (±)-**17**·HCl (isomer 2), 95483-35-5; (±)-**18** (isomer 1), 95483-05-9; (±)-**18** (isomer 2), 95483-36-6; (±)-**18**·H_3PO_4 (isomer 1), 95483-06-0; (±)-**18**·h_3PO_4 (isomer 2), 95483-37-7; **19**, 5447-02-9; (±)-**20**, 95483-07-1; (±)-**21**, 95483-08-2; (±)-$_2$1·HCl, 95483-17-3; **23**, 590-90-9; **25**, 21856-89-3; **26**, 95483-09-3; **27**, 95483-10-6; **28**, 95483-11-7; **29**, 95483-12-8; **30**, 95483-13-9; **31**, 95483-14-0; **32**, 95483-15-1; **33**, 82125-92-6; $CH_3(CH_2)_3NCO$, 111-36-4; *p*-$CH_3C_6H_4CONCO$, 5843-46-9; (±)-norepinephrine, 138-65-8; *p*-toluoyl chloride, 874-60-2; (±)-norepinephrine hydrochloride, 55-27-6; *N*-(2-butyl)norepinephrine, 95483-16-2; 5-hydroxy-2-pentanone, 1071-73-4; 3-hydroxypropylamine, 156-87-6; *p*-tolyl isocyanate, 622-58-2; cyclohexyl isocyanate, 3173-53-3; *p*-tolylsulfonyl isocyanate, 4083-64-1.

Supplementary Material Available: The HPLC parameters and 360-MHz ^1H NMR data for compounds 8–18 (4 pages). Ordering information is given on any current masthead page.

New Antihistaminic Theophylline or Theobromine Derivatives

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A series of 3,4-dihydro-1,3-dimethyl-7-[3-(4-substituted-piperazin-1-yl)-substituted-alkyl]-1*H*-purine-2,6-diones and 3,7-dihydro-3,7-dimethyl-1-[3-(4-substituted-piperazin-1-yl)-substituted-alkyl]-1*H*-purine-2,6-diones was synthesized and evaluated for antihistaminic activity. Some of them displayed good inhibition of both histamine-induced bronchospasm in the anesthetized guinea pig at 10 μg/kg by the intravenous route and of passive cutaneous anaphylaxis in the rat at 10 mg/kg by the oral route. Comparison of the two most active compounds revealed a higher antihistaminic activity with the compounds containing a (phenylthio)propyl group (**1** and **2**) as compared with that containing a phenoxy group. Compound **2** [RS-49014, 3,4-dihydro-1,3-dimethyl-7-[3-[4-[3-(phenylthio)propyl]piperazin-1-yl]-2-hydroxypropyl]-1*H*-purine-2,6-dione] was selected for clinical trials on the basis of a comparative pharmacological study with chloropheniramine, ketotifen, promethazine, and theophylline.

Theophylline and its derivatives are well-known for their bronchodilator activity and consequent efficacy in the treatment of asthma. Related N-7 substituted theophylline derivatives such as caffeine, etofylline, proxyphylline, and reproterol (I) have also been extensively studied.

On the basis of a previous study[1] carried out in our laboratory, we reported that N,N′-disubstituted pipera-

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(1) Beranger, S.; Pinhas, H. French Patent 78.13.114.

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