Functionalized Congeners of 1,3-Dialkylxanthines: Preparation of Analogues with High Affinity for Adenosine Receptors

Kenneth A. Jacobson, **Kenneth L. Kirk**, **William L. Padgett**[†], and **John W. Daly**[†]
Laboratory of Chemistry and Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205.

Abstract

A series of functionalized congeners of 1,3-dialkylxanthines has been prepared as adenosine receptor antagonists. On the basis of the high potency of 8-(*p*-hydroxyphenyl)-1,3-dialkylxanthines, the parent compounds were 8-[4-[(carboxymethyl)oxy]phenyl] derivatives of theophylline and 1,3-dipropylxanthine. A series of analogues including esters of ethanol and *N*-hydroxysuccinimide, amides, a hydrazide, an acylurea, and anilides were prepared. The potency in blocking A_1-adenosine receptors (inhibition of binding of N^6-[^{3}H]cyclohexyladenosine to brain membranes) and A_2-adenosine receptors (inhibition of 2-chloroadenosine-elicited accumulations of cyclic AMP in brain slices) was markedly affected by structural changes distal to the primary pharmacophore (8-phenyl-1,3-dialkylxanthine). Potencies in the dipropyl series at the A_1 receptor ranged from K_I values of 1.2 nM for a congener with a terminal amidoethyleneamine moiety to a K_I value of 58 nM for the parent carboxylic acid to a K_I of 96 nM for the bulky ureido congener. Certain congeners were up to 145-fold more active at A_1 receptors than at A_2 receptors. Various derivatives of the congeners should be useful as receptor probes and for radioiododination, avidin binding, and preparation of affinity columns.

Alkylxanthines, with theophylline (**1**) as the prototype, represent the major class of antagonists for adenosine receptors.[1] Although theophylline and caffeine are relatively weak adenosine antagonists, with affinity constants in the 10–50 μM range, they undoubtedly owe many of their pharmacological effects to blockade of adenosine-mediated functions. Two classes of adenosine receptors have been characterized.[1] The A_1-adenosine receptor is inhibitory to adenylate cyclase and appears involved in antilipolytic, cardiac depressant, and central depressant effects of adenosine analogues. The A_2-adenosine receptor is stimulatory to adenylate cyclase and appears involved in hypotensive, vasodilatory, antithrombotic, and endocrine effects of adenosine analogues. Xanthines, by blockade of adenosine binding at such receptors, would have the opposite effects. Some xanthines, such as 3-iso-butyl-1-methylxanthine, not only block adenosine receptors but also have potent inhibitory effects on phosphodiesterases.[2a] The presence of an 8-phenyl group greatly enhances the potency of theophylline as an adenosine antagonist.[2a,b] Even more potent antagonists result from the replacement of the 1,3-methyl groups of 8-phenyl-theophylline with *n*-propyl groups and by situating uncharged electron-donating para substituents on the 8-phenyl ring. Affinity constants (K_i) of less than 1 nM at A_1-adenosine receptors have been attained.[3,22] In addition to high potency, some selectivity toward the A_1 subclass of adenosine receptors has been obtained with 1,3-dipropyl-8-phenylxanthines.[4]

1,3-Dipropyl-8-(p-hydroxyphenyl)xanthine (**2b**) was chosen as a suitable lead compound in an effort to develop potent and selective functionalized congeners as antagonists for adenosine receptors. An N^6-phenyladenosine was chosen as a suitable lead compound for a parallel effort to develop potent and selective agonists for A_1-adenosine receptors.[5] In the design of active, covalent conjugates of drugs, the goals of the functionalized congener approach are several, including increasing the potency, prolonging the duration of action, and/or changing the specificity. This approach has been applied to isoproterenol and has lead to analogues with activity at β-adrenergic receptors as much as 4 orders of magnitude greater than that of isoproterenol.[6–8] Nontherapeutic applications of active, functionalized drugs include receptor probes,[5,9,10] immobilized ligands for affinity chromatography,[11] and radio-labeled analogues.

We report here the synthesis of a series of highly potent analogues of theophylline and 1,3-dipropylxanthine, some of which contain groups designed for radiolabeling through the introduction of radioisotopes of iodine. The functionalized congeners are also suitable for the preparation of affinity columns. A biotinylated conjugate is intended for use as a molecular probe through linkage to avidin.[12a] Recently, biotinylated derivatives of peptide hormones have been described.[12b,c]

Results and Discussion

Synthetic Methods

The carboxylic acid congener of theophylline (**8a**) and the dipropyl analogue (**8b**) were synthesized by a standard approach[4,13,14] to xanthines as shown in Figure 1. 5,6-Diamino-1,3-dimethyluracil (**3a**) was commercially available, but the 1,3-dipropyl compound (**3b**) was prepared with dipropylurea and cyanoacetic acid as the starting material as described previously.[13,14] The imidazole ring was formed by oxidative closure of the benzylidene adduct 6 derived from the appropriate diaminouracil and the substituted benzaldehyde **4**. The latter was the product of alkylation of p-hydroxybenzaldehyde by iodoacetate.

Ring closure of the benzylidene adduct **6** was effected by heating with substoichiometric amounts of anhydrous ferric chloride.[14] In the case of the dipropyl derivative **6b**, considerable ethyl ester **10** formed in this reaction when ethanol was the solvent. To obviate separating the mixture of acid and ethyl ester, the esterification was brought to completion by prolonged heating of the reaction mixture in the presence of 1 equiv of ferric chloride. In contrast, use of trifluoroethanol as the solvent during ring closure produced **8b** exclusively. Compound **8b** also was prepared by basic hydrolysis of the ester **10**.

Coupling of the carboxylic acid congeners to amines using carbodiimides presented problems due to limited solubility. Attempts to couple **8b** to various polar amines using carbodiimides in dimethylformamide often resulted in isolation of the *N*-acylurea **11** derived from the acid and the coupling reagent. Compound **8a** was coupled in low yield to p-toluidine. In an alternate approach to amide formation (Figure 2), the *N*-hydroxysuccinimide ester **12** of the carboxylic acid congener was prepared and proved to be readily separable from the *N*-acylisourea by crystallization. The *N*-hydroxysuccinimide ester **12** of the carboxylic acid congeners provided an activated form of the drug for coupling to amines (see below) or to bio-polymers such as proteins, the latter then serving as drug carriers.

As an alternative to coupling the preformed xanthine congeners, the amide bond was introduced on the substituted benzaldehyde (e.g., **5**, Figure 1) prior to formation of the imidazole ring as above.

The ethyl ester **9** could be aminolyzed by excess unhindered amines in dimethylformamide to form amides **13** as shown in Figure 2. Aminolysis by alkyldiamines produced the functionalized amino congeners **13c,d**. Compound **13c**, derived from ethylenediamine, was the basis for further chain elongation. The primary amine was readily acylated by *N*-hydroxysuccinimide esters to form amides **14**. A tertiary amine 16 was made via reductive amination sequentially with *p*-hydroxybenzaldehyde and acetaldehyde. The iminium salt **15** was isolated and reduced with sodium cyanoborohydride (Figure 3).

Biological Activity

8-(*p*-Hydroxyphenyl)theophylline and 1,3-dipropyl-8-(*p*-hydroxyphenyl)theophylline, the model compounds chosen for functionalization, have been shown in earlier studies to be potent antagonists of A_1- and A_2-adenosine receptors.[3,4] 8-(*p*-Hydroxyphenyl)theophylline (**2a**) is 280-fold more potent than theophylline in displacing [^{3}H]cyclohexyladenosine from A_1-adenosine receptors in rat cerebral cortical membranes and is 107-fold more potent than theophylline in antagonizing A_2-adenosine receptor mediated activation of cyclic AMP generation by 2-chloroadenosine in guinea pig cerebral cortical slices.[4] Replacement of the 1,3-dimethyl groups of the theophylline analogue **2a** with *n*-propyl groups yields 1,3-dipropyl-8-(*p*-hydroxyphenyl)xanthine (**2b**). This analogue is an extremely potent A_1-adenosine antagonists with a K_i value vs. [^{3}H]cyclohexyladenosine binding in rat cerebral cortical slices of 2.9 nM. The change in the alkyl residues, thus, has increased potency at A_1 receptors by about 17-fold. The change in alkyl residues also increases potency at A_2 receptors but to a much lesser extent (2.6-fold), yielding a somewhat selective A_1 antagonist.

Functionalization of these two xanthines was based on the presence of a *p*-(carboxymethyl)oxy residue on the 8-phenyl ring. This functionalization permitted facile synthesis of a wide variety of amides. In the case of the 8-phenyltheophyllines, the *p*-(carboxymethyl)oxy compound **8a** has a 10-fold lower potency than the *p*-hydroxy compound at A_1 receptors and a 3.3-fold lower activity at A_2 receptors (Table I). It appears likely that the presence of the anionic carboxyl group is not favorable to high affinity binding to either receptor. A similar result was reported for a series of carboxy-functionalized adenosine receptor agonists.[5] The derivative of 8-phenyltheophylline having an anionic *p*-carboxyl group directly on the phenyl ring was even less potent with K_i values of 3000 nM at A_1 receptors and 2500 nM at A_2 receptors.[4] Blocking the carboxylate as an amide as in compound **9a** enhanced receptor potency at both receptor subtypes.

Further syntheses of functionalized congeners were based on the anticipation that the higher potency and selectivity of 1,3-dipropyl-8-(*p*-hydroxyphenyl)xanthine relative to the l,3-dimethyl homologue would be paralleled by higher activity in the series of *p*-(carboxymethyl)oxy derivatives. The *p*-(carboxymethyl)oxy compound **8b**, itself, was 20-fold less potent than the *p*-hydroxy compound **2b** at A_1 receptors. At A_2 receptors the *p*-(carboxymethyl)oxy compound was nearly equipotent with the *p*-hydroxy compound. Again it appears likely that the presence of the anionic carboxy group mitigates against high affinity at A_1 receptors. In an earlier study,[4] 8-(*p*-carboxyphenyl)-1,3-dipropylxanthine was found to be about 60-fold less potent than the *p*-hydroxy compound at A_1 receptors, while being only 2-fold less active at A_2 receptors.[4] The ethyl ester 10 has potency at A_1 and A_2 receptors nearly identical with that of the anionic carboxylic acid **8b**, a finding that does not support the proposal that the carboxylate moiety is responsible for the diminished affinity. The ester of *N*-hydroxysuccinimide **12** is 6.4-fold more potent than the anionic carboxylate **8b** at the A_1 receptor, while being similar in activity to **8b** at the A_2 receptor. The carboxamide **13a** has high affinity at A_1 receptors and is moderately selective, being 8-fold more potent at A_1 receptors than at A_2 receptors. Remarkably, the *p*-toluide **9b** was no more

potent than the acid at A_1 receptors, while being 22-fold less active than the acid at A_2 receptors. This finding stands in direct contrast to results obtained with the analogous compounds in the theophylline (1,3-dimethyl) series. In that series the *p*-toluide **9a** was about 20-fold more potent than the acid **8a** both at A_1 receptors and at A_2 receptors. Obviously, contributions to affinity afforded by the 1,3-dialkyl substituents and by para substituents on the 8-phenyl ring are not independent and can greatly influence each other in either a positive or a negative manner. This fact has been noted in an earlier study on 8-phenyl-1,3-dialkylxanthines.[4] The *p*-hydroxy anilide **9c** was nearly 10-fold more potent than the *p*-toluide **9b** at both A_1 and A_2 receptors. This illustrates the potential contributions of minor structural modifications distant from the primary pharmacophore (in this case the 8-phenylxanthine) to the biological activity.

Long-chain compounds with terminal amino groups had both enhanced A_1 binding and selectivity over the A_2 receptor. The (aminoethyl)amide **13c** was synthesized with a view of increasing water solubility and also of providing a key intermediate for preparation of affinity columns, fluorescent probes, and a biotin-containing xanthine. The (aminoethyl)amide was very potent at A_1-adenosine receptors with a K_i value of 1.2 nM. It was some 40-fold less potent at A_2-adenosine receptors. The solubility of compound **13c** in 0.1 M sodium phosphate, pH 7.2, was 90 μM, compared to 26 μM for compound **13a** and 3.2 μM for compound **2b**. The presence of ethyl and hydroxybenzyl substituents on the terminal amino group (16) had little effect on the potency at A_1 receptors, while reducing potency at A_2 receptors by over 4-fold. This represents the most selective A_1 antagonist (145-fold) in the present series. The analogues in which a functionalized congener is linked to a phenolic moiety were designed for radioiodination. These two compounds are representative of a general approach to radiopharmaceuticals through the attachment of functional moieties into which radioisotopes can be easily introduced.[5]

The acylated compounds **14a** and **14b** were relatively nonselective antagonists for A_1- and A_2-adenosine receptors in contrast to the parent amine, which exhibits a 40-fold selectivity for A_1 receptors. Although less potent than the parent amine, the potencies of the acylated compounds suggest that affinity columns prepared through acyl coupling to the amino compound could be effective in isolation of solubilized A_1 and A_2 receptors and/or xanthine-binding sites. The biotinylated analogue is being investigated as a histochemical probe for adenosine receptors.[12a]

A systematic analysis of the effect of chain length on activity of (aminoalkyl)amides was not carried out. The (aminooctyl) amide **13d** was only 4-fold less potent than the (aminoethyl)amide **13c** at A_1 receptors, while being 10-fold less potent at A_2 receptors. Thus the use of longer spacer chains appear feasible for preparation of affinity columns if the (aminoethyl)amide proves unsatisfactory. Moreover, the high potency of amino congeners of adenosine receptor agonists resembles previous findings of subnanomolar affinity of an adenosine agonist bearing a distal primary amine. This may be due to a favorable interaction with the A_1 receptor, in which an ionic interaction of the distal protonated amine with an anionic moiety of the receptor enhances binding affinity.

One acylurea **11** was prepared and found to have relatively low activity at both A_1 and A_2 receptors. This bulky dicyclohexylurea derivative may exceed the steric limitations for efficient binding to the receptor.

Conclusions

In summary, the functionalized congener approach to xanthine antagonists for adenosine receptors has yielded a series of potent compounds that in some cases are moderately

selective for A_1 receptors. The effects on biological activities caused by modifications of functions distal from the primary pharmcophore in some cases are quite impressive. Similar effects were noted for congeners and conjugates of isoproterenol.[6–8]

Affinities of congeners and derived analogues for the A_1 receptors seem somewhat more sensitive to distal modifications than affinities for the A_2 receptor. As yet no selective A_2-receptor antagonists have been discovered in this study or in earlier studies,[4] and as yet no *specific* A_1-receptor antagonists are available. The present set of functionalized xanthines should prove useful in delineating the role of A_1- and A_2-adenosine receptor blockade in the antiasthmatic,[15] diuretic,[16] respiratory stimulant,[17] central stimulant,[18] cardiac stimulant,[19] analgesic adjuvant,[20] and antiinflammatory[21] activities of theophylline and caffeine.

A further benefit of applying the congener approach to xanthines is the opportunity to increase water solubility. The series of very potent 8-phenylxanthines reported by Bruns et al.[22] was highly nonpolar with aqueous solubility very often falling below 10 μM.[4] By increasing water solubility through the attachment of highly polar groups it should be possible to overcome undesirable binding to plasma proteins and partition into lipids. This could lead to improved pharmacokinetics of xanthine-derived adenosine antagonists.

Experimental Section

Thin-layer chromatography was carried out with use of Analtech silica gel GF plates with mixtures of chloroform/methanol/acetic acid (v/v/v; A, 50/50/5; B, 94/4/2). Reagent grade dimethylformamide (DMF, Aldrich gold label) was stored over 3-Å molecular sieves. Dicyclohexylcarbodiimide (DCC) was purchased from Sigma. 5,6-Diamino-1,3-dimethyluracil hydrate was purchased from Aldrich. The benzylidene adducts of the diaminouracils were prepared by the representative method given for compound **6b**.

Proton NMR spectra were taken on a Varian 220-MHz instrument in the Fourier transform mode. Elemental analyses and mass spectrometry (Finnigan 1015D) were carried out by P. Parisius and N. Whittaker of the Section on Microanalytical Services and Instrumentation of the NIADDK Laboratory of Chemistry. The larger xanthine analogues that did not form molecular ions by chemical-ionization mass spectrometry were identified by Drs. L. Pannell and H. Fales of NHLBI/NIH using californium plasma desorption mass spectrometry.

4-[(Carboxymethyl)oxy]benzaldehyde [(4-Formylphenoxy)acetic Acid, 4]

To a solution of *p*-hydroxybenzaldehyde (49 g, 0.40 mol) in 250 mL of DMF were added iodoacetic acid (75 g, 0.40 mol) and anhydrous potassium carbonate (120 g), and the mixture was stirred at 60 °C for 3 days. The resulting solid was dispersed mechanically in a mixture of ethyl acetate (400 mL) and water (600 mL). The mixture was neutralized cautiously with phosphoric acid. After the dissolution of the solid mass, the neutral aqueous layer was withdrawn. The organic layer was extracted repeatedly with a concentrated solution of dibasic sodium phosphate to remove additional acidic organic material. The aqueous extracts were combined, filtered through glass wool, and acidified to pH 1 with 6 N HCl. This solution was placed in the refrigerator overnight, and a product of tan crystals (21.9 g) was collected. Unreacted *p*-hydroxybenzaldehyde was recovered upon evaporation of the organic layer. The yield based on recovery of starting material was 60%.

4[[(*p*-Toluidinocarbonyl)methyl]oxy]benzaldehyde (5a)

Dicyclohexylcarbodiimide (DCC; 1.32 g, 6.4 mmol) was added to a solution of compound 4 (1.15 g, 6.4 mmol) in tetrahydrofuran (50 mL). After the mixture was stirred for 10 min, *p*-toluidine (0.7 g, 6.5 mmol) was added. After 1 h, the precipitate was removed by filtration, and the filtrate was reduced in volume by evaporation. A crystalline product (1.09 g) was

obtained by recrystallization of the concentrated filtrate from ethyl acetate-/petroleum ether. In order to obtain analytically pure material, it was necessary to remove a higher R_f impurity by thin-layer chromatography (solvent B). This impurity proved to be the imine adduct of the product with p-toluidine (CHN analysis). NMR of **5a** (ppm, Me$_2$SO-d_6): 9.90 (s, 1 H, CHO), 7.91 and 7.15 (each d, 2 H, O-phenyl, J = 8.7 Hz), 7.51 and 7.20 (each d, 2 H, N-phenyl, J = 8.8 Hz), 4.85 (8, 2 H, CH$_2$), 2.27 (s, 3 H, CH$_3$).

4-[[(4-Hydroxyanilino)carbonyl]methoxy]benzaldehyde (5b)

Compound **4** (1.80 g, 10 mmol) was dissolved in 25 mL of tetrahydrofuran containing 20% DMF. To this solution were added DCC (2.06 g, 10 mmol) and after 10 min a solution of p-aminophenol hydrochloride (1.46 g, 10 mmol) and triethylamine (0.78 g, 10 mmol) in DMF (10 mL). After 2 h the precipitate was removed by filtration and washed with tetrahydrofuran. The combined filtrates were evaporated and triturated with water. A yellow oil separated and crystallized, providing 2.40 g of **5b**. Recrystallization from ethanol/petroleum ether gave an analytical sample.

6-Amino-1,3-dipropyl-5-[[4-[(carboxymethyl)oxy]-benzylidene]amino]uracil (6b)

Compound **4** (1.51 g, 8.37 mmol) was dissolved in a mixture of methanol (35 mL) and acetic acid (5 mL) in a 250-mL boiling flask on a steam bath. To this was added a methanolic solution (60 mL) of freshly synthesized 5,6-diamino-1,3-dipropyluracil[10] (**3b**). After the mixture was heated for 15 min, the volume was reduced by evaporation until crystallization occurred. Ether (40 mL) was added and the nearly white solid was collected to give 2.80 g. Mass spectrum (CI-NH$_3$), m/z 389 (s), 331 (w), 329 (w), 227 (s), 212 (s), 167 (9).

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-dimethylxanthine (8a)

The benzylidene adduct between compound **4** (0.609 g, 3.38 mmol) and 5,6-diamino-1,3-dimethyluracil hydrate (0.58 g, 3.4 mmol) was prepared as above for the synthesis of **6b**. Tan crystals (0.963 g, 85.7%) were obtained upon cooling the reaction mixture overnight in the refrigerator. The benzylidene adduct (98 mg), used without further purification, was dissolved in warm DMF (7 mL), treated with ferric oxide (20 mg), and heated on the steam bath for 4 h. After addition of an equal volume of ethanol, the precipitate was collected and dried to give 76 mg of **8a** (67% overall yield from **4**).

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-dipropylxanthine (8b)

Method A—The benzylidene adduct **6b** (191 mg, 0.49 mmol) was suspended in trifluoroethanol (15 mL) and dissolved by refluxing on a steam bath. Anhydrous ferric chloride (20 mg) was added, and heating was continued for 2 h. Ether was added to complete the precipitation of product, which was collected and dried in vacuo. The crude product, 0.17 g, was recrystallized from DMF/methanol/ether to give chromatographically pure **8b**.

Method B—The ethyl ester 10 (114 mg, 0.28 mmol) was dissolved in DMF (5 mL) and treated with aqueous 0.1 N sodium carbonate (5 mL). The mixture was heated on the steam bath for 0.5 h. The solvent was evaporated, leaving a white film, which was triturated with dilute HCl. The resulting white precipitate was collected and washed with water and dried in vacuo. This material (105 mg) was homogeneous by TLC (solvent B; R_f 0.42) and identical with **8b** prepared by method A.

8-[4-[[(p-Toluidinocarbonyl)methyl]oxy]phenyl]-1,3-dipropylxanthine (9b)

The *p*-toluide of the carboxylic acid congener **8b** was prepared by the method described below for compound **9c**, except that the reaction was continued overnight.

8-[4-[[[(4-Hydroxyanilino)carbonyl]methyl]oxy]-phenyl]-1,3-dipropylxanthine(9c)

The benzylidene adduct between freshly prepared compound **3b**[13,14] (0.385 mmol) and the substituted benzaldehyde **5b** (88 mg, 0.325 mmol) was formed according to the method described for compound **6b**. The solid adduct (0.14 g, 90% yield) was dissolved in hot absolute ethanol (10 mL), treated with ferric chloride (20 mg), and heated on the steam bath until the product precipitated (30 min). Ether was added, and the product (93 mg, 60% overall yield from compounds **3b** and **4**) was collected by filtration.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-dipropylxanthine Ethyl Ester (10)

Compound **6b** (1.69 g, 4.3 mmol) was suspended in 100 mL of absolute ethanol. Anhydrous ferric chloride (0.70 g, 4.3 mmol) was added, and the mixture was refluxed on a steam bath for 1 day. The slow conversion of the free acid (identical with compound **8b**, R_f 0.35) to the ethyl ester (R_f 0.78) was followed by TLC on silica gel using solvent B. The reaction mixture was evaporated in vacuo to a small volume, and dry diethyl ether was added. The bulky crystalline mass was collected by filtration, washed with ether, and dried in vacuo to give 1.27 g of 10.

N-[[[[4-(1,3-Dipropyl-8-xanthinyl)phenyl]oxy]methyl]-carbonyl]-N,N'-dicyclohexylurea (11) and 8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-dipropylxanthine N-Hydroxysuccinimide Ester (12)

The carboxylic acid congener **8b** (18.4 mg, 0.048 mmol) was dissolved in DMF (5 mL), cooled in an ice bath, and treated with *N*-hydroxysuccinimide (6 mg) and DCC (11 mg). After the mixture was stirred for 1 day at room temperature, the urea was removed by filtration. Upon addition of water a white solid precipitated and was collected. Recrystallization from DMF/water provided 11.1 mg of pure 12. A side product removed by crystallization proved to be the *N*-acylurea 11.

The identical urea 11 was isolated as an undesired product in the attempted DCC coupling in DMF/water of the carboxylic acid **6b** with highly polar amines.

8-[4[[[[(2-Aminoethyl)amino]carbonyl]methyl]oxy]-phenyl]-1,3-dipropylxanthine (13c)

Compound **10** (57.5 mg, 0.14 mmol) was dissolved in warm DMF (1.0 mL). When the solution reached room temperature, ethylenediamine (1.0 mL) was added. After the solution was stirred overnight, most of the solvent was evaporated under a stream of nitrogen. The resulting oil was triturated with methanol. After crystallization began, ether was added, and the product was collected and dried to give 59 mg of **13c**, homogeneous by TLC (solvent system A): UV peaks at 309 and 248 nm.

8-[4-[[[[[2-(Biotinylamino)ethyl]amino]carbonyl]-methyl]oxy]phenyl]-1,3-dipropylxanthine (14b)

Compound **13c** (24.1 mg, 0.056 mmol) was suspended in 1 mL of DMF. *N*-Succinimidyl-*d*-biotin (Sigma, 23.6 mg, 0.069 mmol) was added with stirring. A solution formed after several minutes, and a precipitate appeared soon thereafter. After 1 day methanol (1 mL) and diethyl ether were added. The precipitate was collected and dried to give 26.6 mg of **14b.**

8-[4-[[[[2-[(4-Hydroxybenzylidene)ammonio]ethyl]-amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine Acetate (15)

Compound **13c** (56 mg, 0.13 mmol) and 4-hydroxybenzaldehyde (19 mg, 0.16 mmol) were dissolved in warm acetic acid (5%) in ethanol (2 mL) and heated on a steam bath for 2 h. The solvent was evaporated and the residue triturated with ether to give **15** as a tan solid: NMR (ppm, Me_2SO-d_6) 8.15 (s, 1 H, CH=N), 8.05 and 7.04 (each d, 2 H, 8-phenyl, $J = 8.9$ Hz), 7.55 and 6.78 (each d, 2 H, phenol, $J = 8.5$ Hz), 4.56 (s, 2 H, CH_2O), 3.59 (CH_2N), 1.91 (s, 3 H, acetate), and signals from propyl groups (Table III).

8-[4-[[[[2-[Ethyl(4-hydroxybenzyl)ammonio]ethyl]-amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine Acetate (16)

Compound **15** (8.7 mg, 0.015 mmol) was suspended in methanol (1 mL) and treated with excess sodium cyanoborohydride (20 mg, 0.32 mmol). The mixture was warmed at 60 °C to form a solution and treated with acetaldehyde (0.03 mL). After 2 h the solvent was evaporated and the residue was chromatographed on LH-20 eluting with methanol. Evaporation of the solvent left a clear film of 16 (5.9 mg, 61%). The product was chromatographically pure (R_f 0.45, Analtech RPS-F, 75% MeOH/5% HOAc/H_2O, positive Pauly reaction, unreactive toward ninhydrin). An average molecular weight of 563 was determined by californium plasma desorption mass spectroscopy.

Biochemical Assays

Inhibition of binding of 1 nM N^6-[^{3}H]cyclohexyladenosine to A_1-adenosine receptors in rat cerebral cortical membranes was assayed as described.[24] Inhibition of binding by a range of concentrations of each xanthine was assessed in triplicate for at least two separate experiments. Inhibition of 2-chloroadenosine-stimulated cyclic AMP accumulation in [^{3}H]adenine-labeled guinea pig cerebral cortical slices was assayed essentially as described.[24] In the present experiments 10 μg/mL of adenosine deaminase was present in incubations with slices to prevent effects of endogenous adneosine, and 30 μM 4-[3(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone (rolipram, ZK 62711)[25] was present to inhibit phosphodiesterases. Under these conditions 2-chloroadenosine elicited a maximal 10–20-fold increase in levels of radioactive cyclic AMP in guinea pig cortical slices with an EC_{50} of aboout 8 μmol (data not shown). Inhibition of the response to 15 μM 2-chloroadenosine by a range of concentrations of each xanthine was assessed in triplicate in at least two separate experiments.

Acknowledgments

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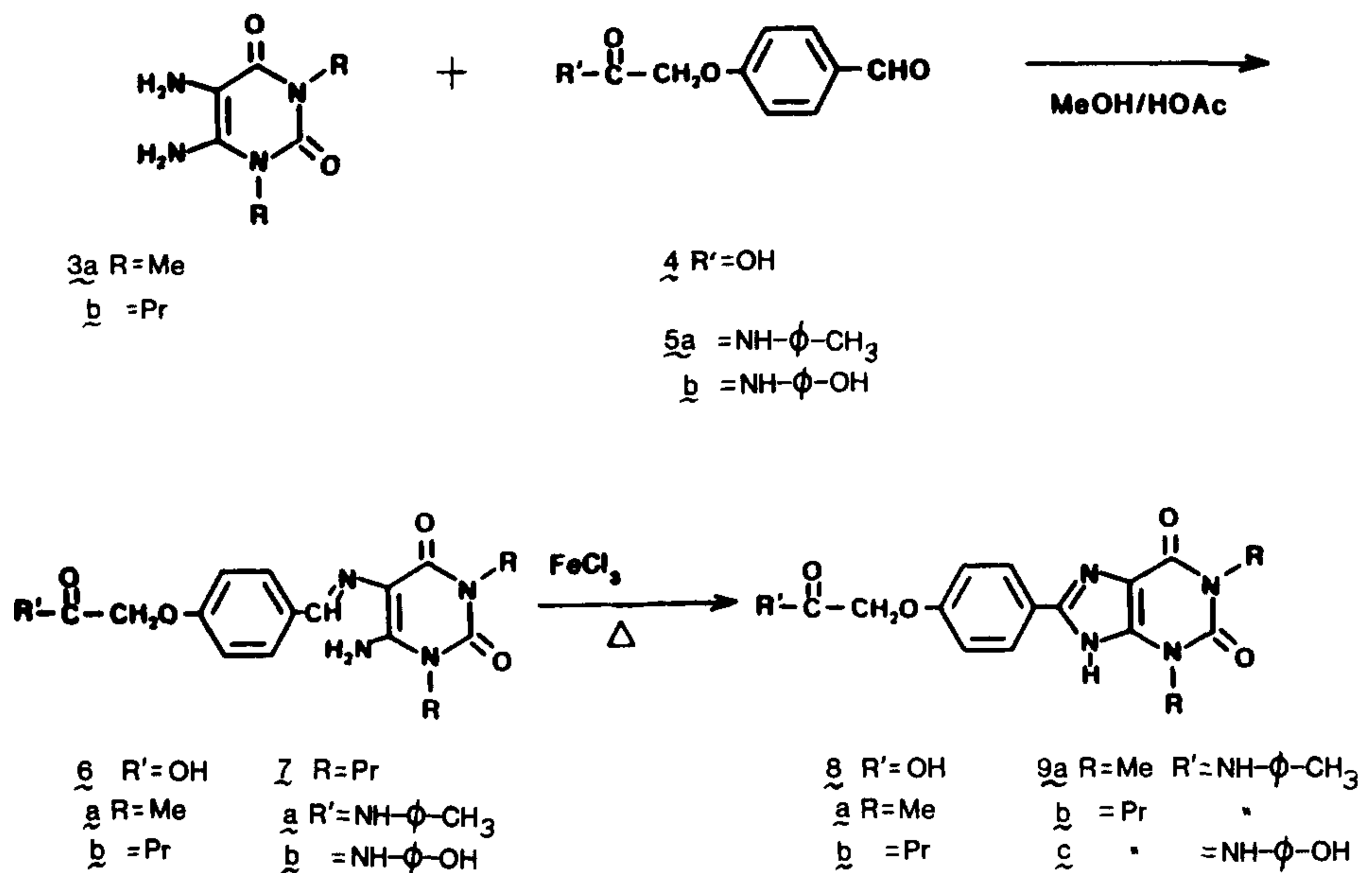
Figure 1.
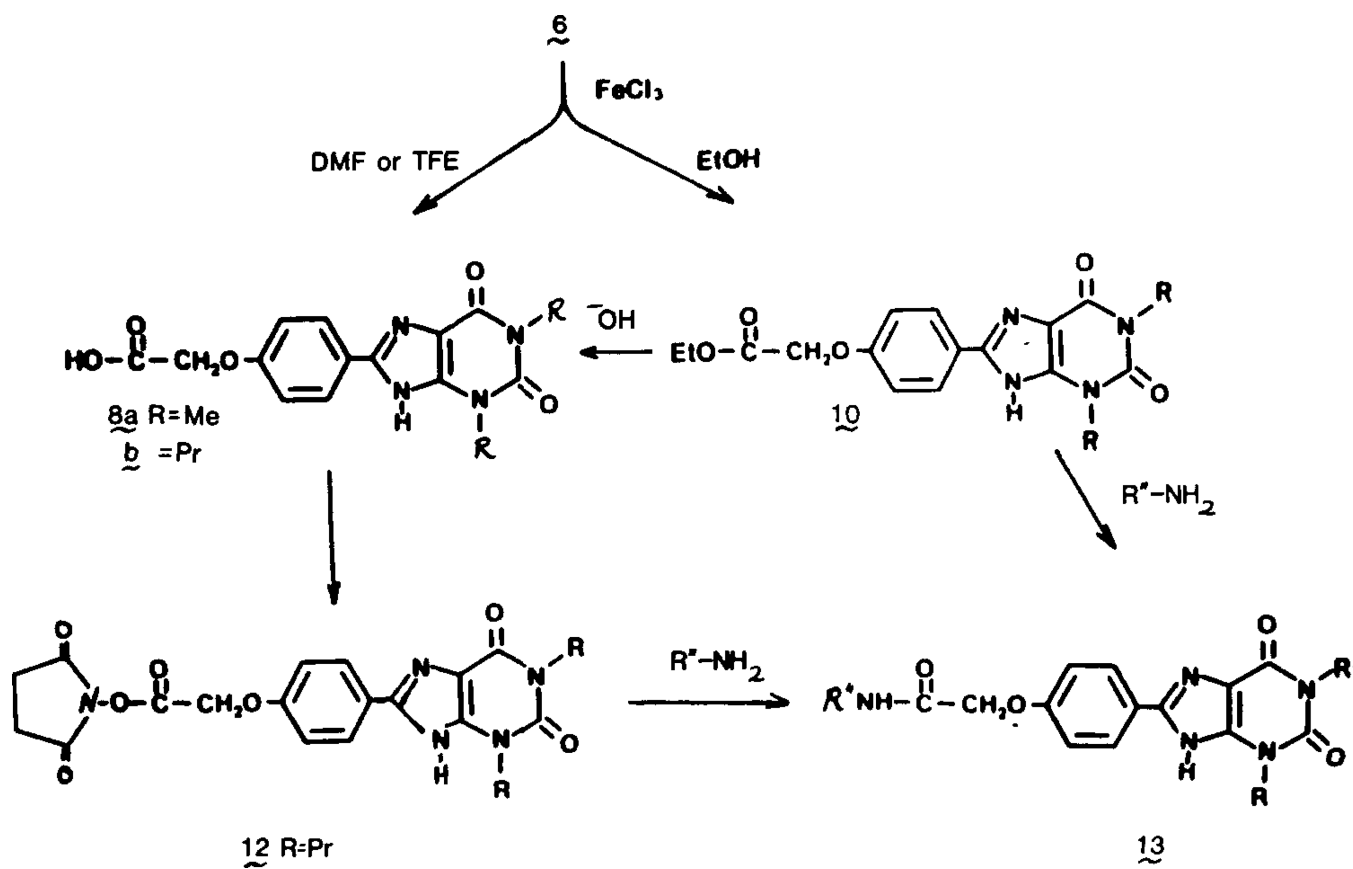Synthetic approach to functionalized xanthine derivatives.

Figure 2.
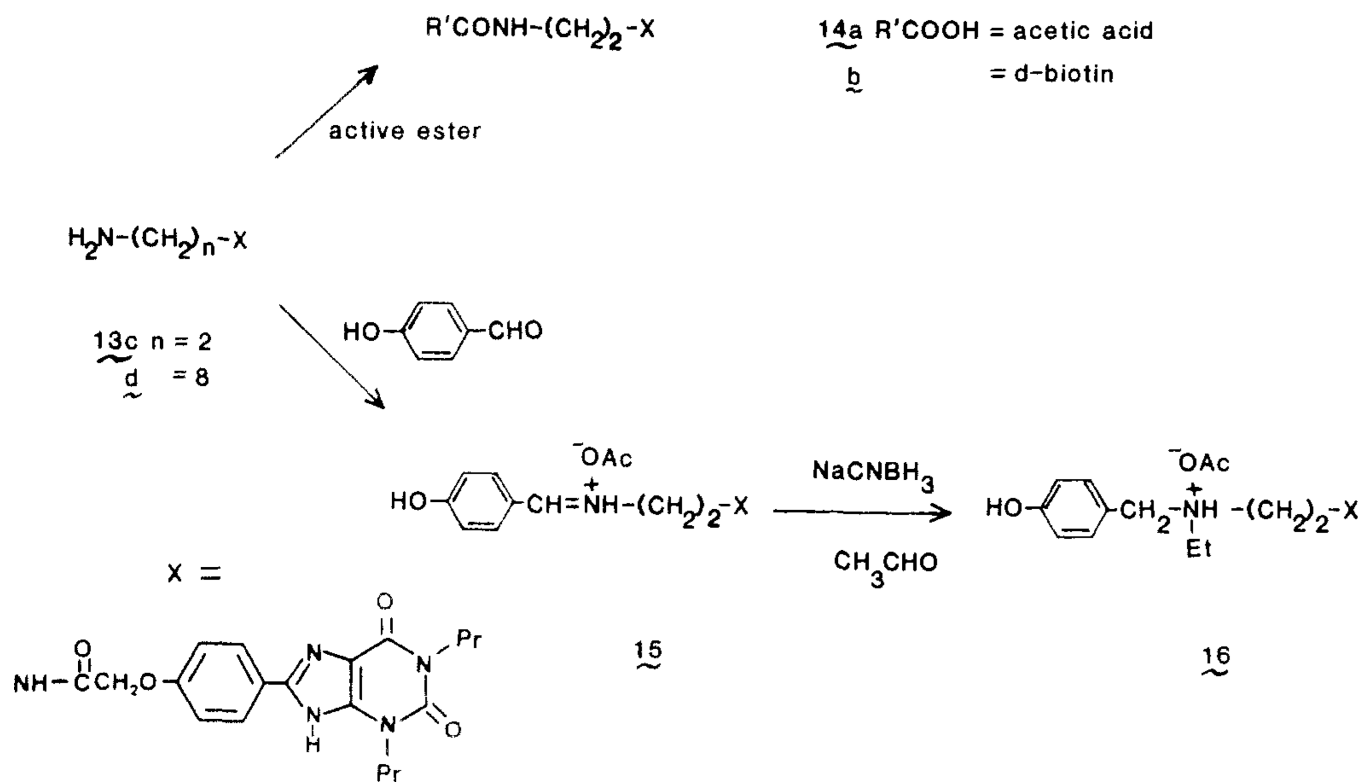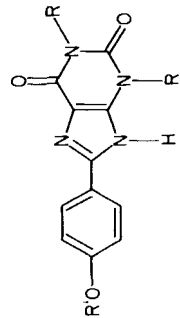Methods of coupling and chain elongation of functionalized xanthines.

$R'CONH-(CH_2)_2-X$

14a R'COOH = acetic acid
b = d-biotin

active ester

$H_2N-(CH_2)_n-X$

13c n = 2
d = 8

HO—⟨ ⟩—CHO

X =

HO—⟨ ⟩—CH=NH⁺-(CH_2)_2-X   ⁻OAc $\xrightarrow{\text{NaCNBH}_3}$ HO—⟨ ⟩—CH_2-NH⁺-(CH_2)_2-X   ⁻OAc
                                                    CH_3CHO Et

15

16
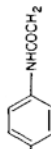
Figure 3.
Reactions of amino-functionalized xanthines.

Table I

Effect of Functionalization on the Activity of 1,3-Dialkyl-8-(*p*-hydroxyphenyl)xanthines at A_1- and A_2-Adenosine Receptors

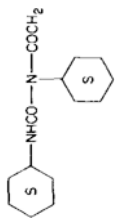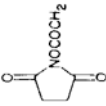| compd	R′	$K_i,^a$ nM		
		A_1 receptor	A_2 receptors	ratio A_1/A_2
R = CH$_3$				
2a	H	50 ± 20	130 ± 30	2.6
8a	HO$_2$CCH$_2$	500 ± 200	430 ± 80	0.86
9a		26 ± 5	20 ± 7	0.77
R = *n*-C$_3$H$_7$				
2b	H	2.9 ± 0.8	50 ± 10^b	17
8b	HO$_2$CCH$_2$	58 ± 3	34 ± 13	0.59
9b		36 ± 23	750 ± 370	21
9c		4.1 ± 1.5	62 ± 37	15
10	CH$_3$CH$_2$OCOCH$_2$	42 ± 3	30 ± 12	0.71
11		96 ± 25	> 1000 (30% inhibn)	> 10
12		9.0 ± 0.7	40 ± 14	4.4
13a	H$_2$NCOCH$_2$	6.0 ± 1.0	47 ± 2	7.8
13b	(CH$_3$)$_2$ NCOCH$_2$	32 ± 7	68 ± 39	2.1

compd	R′	A$_1$ receptor	A$_2$ receptors	ratio A$_1$/A$_2$
			$K_i,{}^a$ nM	
13c	H$_2$N(CH$_2$)$_2$NHCOCH$_2$	1.2 ± 0.5	49 ± 17	41
13d	H$_2$N(CH$_2$)$_8$NHCOCH$_2$	5 ± 2	470 ± 170	94
13e	H$_2$NNHCOCH$_2$	59 ± 0.7	32 ± 4	0.54
14a	CH$_3$CONH(CH$_2$)$_2$NHCOCH$_2$	24 ± 3.5	62 ± 3	2.6
14b	(CH$_2$)$_4$CONH(CH$_2$)$_2$NHCOCH$_2$	54 ± 2	180 ± 80	3.3
16	CH$_2$N(CH$_2$)$_2$NHCOCH$_2$, CH$_2$CH$_3$·HOAc	2.2 ± 0.7	320 ± 70	145

[a]IC$_{50}$ values for A$_1$ receptors were obtained from antagonism of binding of 1 nM [^{3}H]cyclohexyladenosine to rat cerebral cortical membranes. IC$_{50}$ values for A$_1$ receptors were obtained from antagonism of ^{3}H-labeled cyclic AMP accumulation elicited by 15 µM 2-chloroadenosine in [^{3}H]adenine-labeled guinea pig cerebral cortical slices. K_i values were calculated from the equation $K_i = IC_{50}/$ (1 + concentration of adenosine analogue/K_a for adenosine analogue). Values are means ± SEM for two to four experiments. Each experiment contained triplicate determinations.
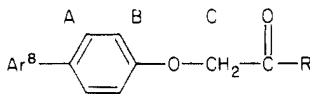
[b]Values from ref 4.

Table II

Analytical Data

compd	method	% yield	mp, °C	formula	anal.	MS[f]
4	a	60	191–193	$C_9H_8O_4$	C, H	209, 181, 135[g]
5a	b	63	124–126	$C_{16}H_{15}NO_3$	H, N; C[l]	270[g]
5b	b	89	185–186	$C_{15}H_{13}NO_4$	C, H, N	272[g]
6b	a	86	179–180	$C_{19}H_{24}N_4O_5$	C, H, N	389
8a	c	67	>310	$C_{15}H_{14}N_4O_5 \cdot 1/2 Me_2SO$	C, H, N	331, 272
8b	c	77	283–285	$C_{19}H_{22}N_4O_5$	C, H, N	387, 329
9a	a	22	287–290	$C_{22}H_{21}N_5O_4 \cdot 1/2 H_2O$	C,H, N	420, 272
9b	c	32	>300	$C_{26}H_{29}N_5O_4 \cdot 5/4 H_2O$	C, H, N	476[i]
9c	c	60	>320	$C_{25}H_{27}N_5O_5 \cdot H_2O$	C, H, N	478[i]
10	a	71	243–244	$C_{21}H_{26}N_4O_5$	C, H, N	415[g]
11	a	63	d < 190[j]	$C_{32}H_{44}N_6O_5 \cdot 1/2 H_2O$	C, H, N	593[i]
12	a	48	241–245	$C_{23}H_{25}N_5O_7 \cdot 1/2 H_2O$	C, H, N	386,[h] 329
13a	d	62[k]	301–303	$C_{19}H_{23}N_5O_4$	H, N; C[m]	386, 329
13b	d	68[k]	227–231	$C_{21}H_{27}N_5O_4$	n	414, 329
13c	d	99	218–220[d]	$C_{21}H_{28}N_6O_4 \cdot 5/6 H_2O$	C, H, N	429, 411[g]
13d	d	14[k]	> 300	$C_{27}H_{40}N_6O_4$	o	513[i]
13e	d	90	>310	$C_{19}H_{24}N_6O_4 \cdot 7/4 H_2O$	C, H, N	401, 328
14a	e	51	309–312	$C_{23}H_{30}N_6O_5 \cdot 5/4 H_2O$	C, H, N	471[i]
14b	e	50	262–264	$C_{31}H_{42}N_8O_6S \cdot 3/2 H_2O$	C, H, N	655[i]
15	a	75	186–190	$C_{30}H_{36}N_6O_7$	C, H, N	

[a] Refer to Experimental Section.

[b] Carbodiimide coupling as in **5b**

[c] Benzylidene formation and oxidation (overall yield given). Compound **8a** was recrystallized from Me_2SO/ether.

[d] Aminolysis as in **13d.**

[e] N-Hydroxysuccinimide coupling

[f] Chemical ionization, NH3 gas, unless noted.

[g] CH4 gas.

[h] Converted to compound **13a** by ionizing gas.

[i] Californium plasm desoprtion mass spectrometry,[24] positive ions.

[j] Raise temperature rapidly.

[k] Chromatographically homogeneous product obtained after preparative TLC (solvent B).

[l] C: calcd, 71.36; found, 70.80; accurate mass; calcd, 269.1052; found, 269.1040.

[m] C: calcd, 59.21; found, 58.07; accurate mass; calcd, 385.1750; found, 385.1730.

[n] Accurate mass; calcd, 413.2063; found, 413.2076.

[o] Noncrystalline solid; could not be recrystallized.

Table III

NMR Data[a] for Xanthines

compd	A[b]	B[b]	C[c]	R
8a	8.07	7.05	4.77	
8b	8.05	7.05	4.78	
9a	8.12	7.14	4.79	7.52,[b] 7.14,[b] 2.27 (s, 3 H, CH$_3$)
9b	8.10	7.14	4.78	7.53,[b] 7.14,[b] 2.27 (s, 3 H, CH$_3$)
9c	8.13	7.17	4.77	7.45,[b] 6.76[b]
10	8.07	7.08	4.88	4.12 (q, 2 H, CH$_2$), 1.23 (t, 3 H, CH$_3$)
11	8.03	6.86	4.77	5.57 (1 H), 3.5 (1 H), 1.2–1.6 (20 H)
12	8.09	7.14	5.43	2.84 (s, 4 H)
13a	8.07	7.07	4.51	7.58 (s, 1 H, NH), 7.17 (s, 1 H, NH)
13b	8.05	7.04	4.91	3.02 (s, 3 H), 2.86 (s, 3 H)
13c	8.05	7.05	4.55	3.20 (2 H, CH$_2$, 2.68 (2 H, CH$_2$)
13d	8.00	6.98	4.53	3.12 (2 H, CH$_2$), 2.77 (2 H, CH$_2$), 1.2–1.6 (12 H)
13e	8.05	7.08	4.56	9.35 (1 H, NH)
14a	8.08	7.09	4.55	3.1–3.2 (4 H, (CH$_2$)$_2$), 1.79 (s, 3 H, CH$_3$)
14b	8.07	7.04	4.55	6.40[d] and 6.34[d] (each s, 1 H, NH), 4.3[d] and 4.1[d] (each 1 H, CH$_2$NH), 3.17 (4 H, CH$_2$NH), 3.05[d] (1 H, CHS), 2.6[d] and 2.8[d] (each 1 H, CH$_2$S), 2.04[d] (t, 2 H, CH$_2$CO), 1.3–1.5[d] (6 H)
16	8.06	7.1	4.53	7.1 (2 H, Ar), 6.68 (2 H, Ar), 4.34 (s, 2 H, CH$_2$Ar), 3.44, 3.22, and 2.43 (each 2 H, CH$_2$), 1.0 (CH$_3$)

[a] In Me$_2$SO-d_6, δ from Me$_4$Si. Signals from the 1,3-dialkyl groups are not included. Typical values are as follows: 3.51 (s, 3 H), 3.28 (s, 3 H) for compound **9a;** 4.06 (t, 2 H, CαH$_2$), 3.91 (t, 2 H, CαH$_2$), 1.78 (2 h, CβH$_2$), 1.63 (2 H, CβH$_2$), 0.90 (6 H, CH$_3$) for compound **9b.**

[b] Doublet, 2 H, aromatic, J = 8–9 Hz

[c] Singlet, 2 H.

[d] Biotin moiety.