DEIODOTRIFLUOROMETHYLATION OF ETHYL 3,3',5-TRIIODOTHYROACETATE. DIVERGENT DERIVATIZATION BASED ON THE COMBINATORIAL CONCEPT

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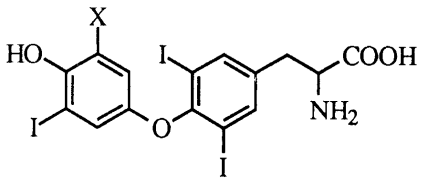
A strategy for development of thyroid hormone analogs was examined based on the combinatorial concept. The reaction of ethyl 3,3',5-triiodothyroacetate (**3b**) with Cd / Br_2CF_2 / CuBr followed by ester hydrolysis gave a mixture of more than ten products as detected by HPLC. The structures of the products in the bioactive fractions showed that the replacement of iodides with trifluoromethyl groups is an effective approach for obtaining thyromimetics.

KEY WORDS divergent derivatization; thyroid hormone; triiodothyroacetic acid; nuclear receptor; combinatorial chemistry; trifluoromethylation

Thyroid hormones, 3,3',5-triiodothyronine (T_3, **1**) and 3,3',5,5'-tetraiodothyronine (T_4, **2**), play significant roles in development, differentiation, and basal metabolism of mammals,[1] and have possible clinical applications for treatment of hypercholesterolemia or obesity, although they have serious cardiac side effects.[2] Among various thyromimetic compounds so far known, only a few show superior therapeutic ratios.[3] The biological actions of thyroid hormones are elicited by the regulation of the expression of specific genes through binding to and activating the thyroid hormone receptors (TRs),[1,4] which belong to the nuclear receptor superfamily.[5] There are two subtypes of TRs (TRα and TRβ), both of which have several isoforms. The complexation of thyroid hormone with these nuclear receptors is the key event in thyroid hormonal action, but the structural requirements of thyroid hormones as ligands of nuclear receptors are still unclear,[6] compared with those of other hydrophobic ligands of nuclear receptors (steroid hormones, retinoic acid, and so on). Furthermore, compounds that have unique binding affinities to each receptor are of potential clinical value, as thyromimetic agents having different pharmacological properties, such as tissue-selectivity.[3] Here, we describe a new methodology, divergent derivatization based on the combinatorial concept (DDCC), illustrated for obtaining thyroid hormone analogs.



1 (T_3, X = H)
2 (T_4, X = I)

Combinatorial chemistry[7] is a potent technique in medicinal chemistry, owing to recent progress in analytical and biochemical methods. Generally, there are two types of methods to get a combinatorial library. One is the reaction of mixtures of building blocks, which may afford libraries of huge size, such as peptide and oligonucleotide libraries. The other is the nonselective reaction of substrates having multiple reactive sites, which would afford a number of products at once, including unexpected products (usually treated as by-products). In order to confirm the usefulness of the latter method (divergent derivatization) for research on new thyromimetics, we took a simple example, that is, the reaction of 3,3',5-triiodothyroacetic acid (Triac) ethyl ester (**3b**), a potent thyroid hormone agonist, with a trifluoromethylating reagent (Fig. 1). Expected products are **4** – **8**, whose iodide(s) have been replaced with trifluoromethyl group(s). For biological assay,

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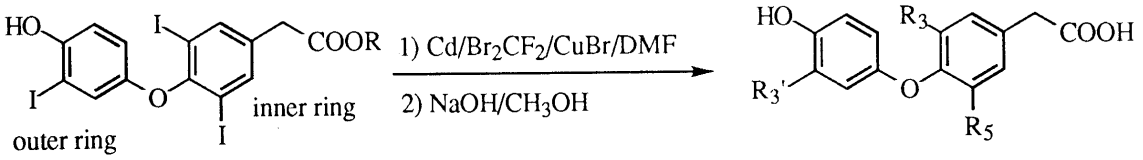

3a (Triac, R = H)
3b (R = C_2H_5)

Fig. 1

4 (R_3 = I, R_5 = I, $R_{3'}$ = CF_3)
5 (R_3 = CF_3, R_5 = I, $R_{3'}$ = I)
6 (R_3 = CF_3, R_5 = I, $R_{3'}$ = CF_3)
7 (R_3 = CF_3, R_5 = CF_3, $R_{3'}$ = I)
8 (R_3 = CF_3, R_5 = CF_3, $R_{3'}$ = CF_3)

we constructed a competitive receptor-binding assay using [^{125}I]**1** and a recombinant fusion protein (MBP-TRα1) which consists of the ligand-binding domain of human TRα1 and maltose-binding protein (MBP).[8] This enabled us to use a microscale assay to examine thyroid hormonal activities.

Triac ethyl ester (**3b**)[9] was treated with trifluoromethyl copper, prepared from cadmium, dibromodifluoromethane and copper bromide in DMF.[10] A typical procedure is as follows. Dibromodifluoromethane (0.7 ml, 7.7 mmol) was slowly added to a suspension of Cd powder (1.38 g) in DMF (4 ml) at 0 °C, and the mixture was stirred for 1.5 h at room temperature. Then, HMPA (4 ml), copper bromide (448.6 mg, 3.0 mmol) and **3b** (205.5 mg, 0.32 mmol) were added successively, and the whole was heated at 60 °C overnight. The mixture was poured into a mixture of 2 N hydrochloric acid and ether (1 : 1), and filtered on Celite. The filtrate was separated, and the organic layer was washed with water and dried over sodium sulfate. The crude mixture (163 mg) was chromatographed on a small amount of silica gel (eluent: ethyl acetate), and then a part of the resultant mixture (12 mg) was treated with base (ester hydrolysis) to afford a mixture of thyromimetic candidates.

The crude acid mixture was analyzed and separated by HPLC (Polygosil 60-5C18, eluent CH_3CN/20 mM aqueous ammonium acetate, pH 4.0). As shown in Fig. 2A, about twenty peaks were detected, besides the major peak f corresponding to Triac (**3a**). Examination of the area around peak f under another HPLC condition afforded another small peak (p, Fig. 2B). The activity was estimated as the inhibition of [^{125}I]**1** binding to MBP-TRα1 by a 10-fold excess of each fraction, each molar amount being approximated by the extinction coefficient of UV (280 nm). Therefore, the bar heights in Fig. 2 show the relative activity of each compound. The binding ability of each fraction (peaks a – p) to MBP-TRα1 showed that most of the activity was present in the region from peak d to peak h. Peak p was inactive in this experiment, but when used in 100-fold excess, it showed a weak (about 15%) inhibition of receptor binding by [^{125}I]**1**.

The structures of active fractions (peaks d – h) were determined by mass spectroscopy and

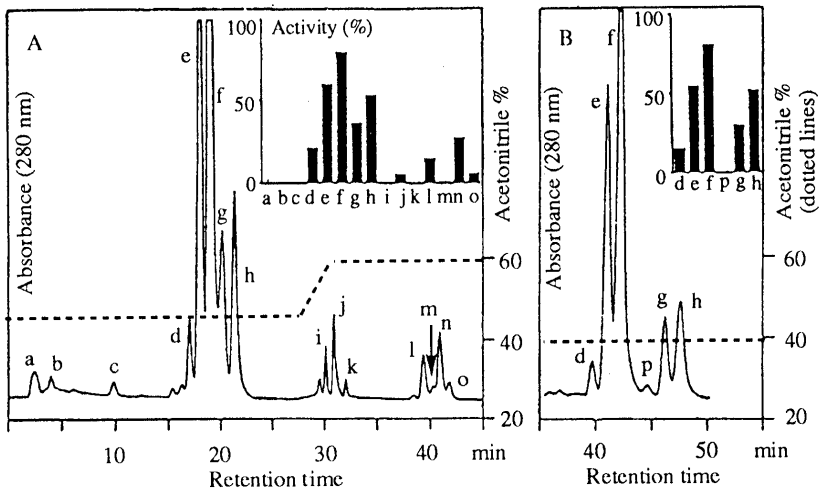

Fig. 2. Separation of Thyromimetic Candidate Library by HPLC and the Competitive Binding Activities of Peaks a –p. Eluent: (A) CH_3CN (46% to 60%)/20 mM NH_4OAc (pH 4.0), (B) 40 % CH_3CN/20 mM NH_4OAc (pH 4.0) for the region of peaks d–h. Dotted lines indicate percentage of CH_3CN. The activity (bars) was estimated as the inhibition of [^{125}I]**1** binding to MBP-TRα1 by a 10-fold excess of each fraction.[8]

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^{1}H-NMR. The observed M^+ (HRMS) indicated that these fractions contained the compounds in which iodine(s) were simply replaced with trifluoromethyl group(s). Finally, these peaks could be assigned as shown in Table 1 by comparison of ^{1}H-NMR chemical shifts. The weakly active fraction (peak p) was assigned as **8** in which all the iodines were replaced with trifluoromethyl groups. The compounds having one trifluoromethyl group instead of iodine on the inner (**5**) or outer ring (**4**) had similar potency to Triac (**3a**). Replacement of two iodines with trifluoromethyl groups decreased the activity by one order of magnitude, as determined by dose-dependency experiments (data not shown). Interestingly, compound **8** with three trifluoromethyl groups was still active, although its potency is weak. The role of iodo atoms in thyroid hormone structures is unclear, but the replacement of iodine by a methyl group (especially on the inner ring) usually causes a significant decrease of the activity. Thus, this finding on the potency of trifluoromethylated compounds suggests some similarity between iodine and the trifluoromethyl group in their electronic effects. Structural studies on the other fractions are also in progress, and should afford more information on the structural requirements for binding ability to TRs.

Table 1. Structural Assignment of Active Fractions (Peaks d – h)

Peak	M^+	Formula (HRMS)	Structure
d	506	$C_{16}H_9O_4F_6I$	**7**
e	564	$C_{15}H_9O_4F_3I_2$	**5**
f	622	$C_{14}H_9O_4I_3$	**3a**
g	506	$C_{16}H_9O_4F_6I$	**6**
h	564	$C_{15}H_9O_4F_3I_2$	**4**
p	448	$C_{17}H_9O_4F_9$	**8**

In conclusion, we have demonstrated an efficient new method for obtaining thyromimetics. Trifluoromethylated analogs of **3a** should be useful tools for studies of thyroid hormonal actions, and for aiding the development of clinical thyromimetics. Furthermore, this simple reaction could be made more complex, for example by using a mixture of reaction substrates or reagents, to get larger libraries. We propose divergent derivatization as an efficient method in medicinal chemistry.

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