New Protective Groups for Peptide Synthesis. 4. Chromone-Derived Protection for Amine and Carboxyl Functions

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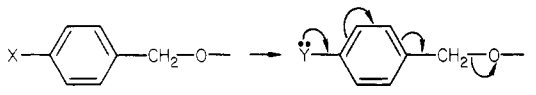
A new urethane-type protective group for amines, the 2-(trifluoromethyl)-6-chromonylmethylenecarbonyl (Tcroc) group (**15**), and a new benzyl ester type protective group for carboxylic acids, the 2-(trifluoromethyl)-6-chromonylmethylene (Tcrom) group (**12**), are described. The Tcroc and Tcrom groups are shown to be rapidly cleaved by neat propylamine or dilute ethanolic hydrazine and to resist cleavage by ethyl alaninate and a variety of acidic reagents. Independent removal of Tcroc or Tcrom and of Boc or *tert*-butyl ester groups, each in the presence of the other, is demonstrated by a synthesis of N^{α}-Boc methionine enkephalin.

Amine and carboxyl protective groups which meet the most exacting requirements of peptide synthesis remain unavailable, despite extensive research which has led to many otherwise useful blocking groups.[1] An ideal blocking group for peptides or proteins must resist repeated operations of purification and synthesis, it must aid solubilization of large peptides, and it must be removable reliably and in virtually quantitative yield under deblocking conditions which leave other protective groups intact and which do not damage the deprotected product.

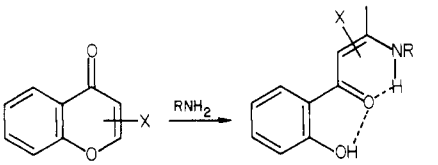
Acids, bases, oxidizing agents, reducing agents, nucleophiles, and light are the categories of reagents that have been used to cleave peptide blocking groups, and of these, reducing agents and nucleophiles appear to us to be the most promising candidates for producing deblocking conditions which are compatible with sensitive peptides or proteins. Previously[2] we have described a general principle for designing blocking groups in which use is made of the extensively documented[3] coupling of rate of heterolytic benzyl–oxygen cleavage with electronic character of a para substituent of the benzyl function. In effect, a function **1** and an agent which can convert the electron-withdrawing group X of **1** to an electron-withdrawing group Y of **2** provides the basis from which one can develop a new protective group. In this paper we report realization of protective groups for carboxyl and amino functions which are based on the reactivity of chromones toward certain nitrogen nucleophiles.



1 **2**

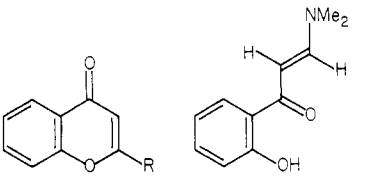
Preliminary Studies

Michael addition followed by ring cleavage to form vinylogous amides **4** occurs if a chromone **3** is heated with primary amines.[4] Since the ring oxygen of **3** is involved with pyrone-type resonance, it is not expected to be a good electron donor; however, the phenolic hydroxyl of **4** is expected to allow the fragmentation shown for **2**.



3 **4**

We were attracted by the expectation that the conversion **3** → **4** would prove to be a reversible process with the direction of equilibrium determined in part by stabilization of **4** by two hydrogen bonds and vinylogous amide resonance. Structural factors that eliminate a hydrogen bond of **4** or that distort its carbonyl and enamine functions from coplanarity are expected to destabilize it relative to the free amine and **3**. By proper choice of pyrone ring substituents, we hoped to find a chromone function which can be converted rapidly and quantitatively to **4** under mild and general conditions and which fails to react with secondary amines or with branched-chain primary amines that are relatively weak bases, such as the N-terminal amino functions of peptides.[5]



5a, R = H
b, R = CH_3
c, R = CF_3

6

Chromone itself, **5a**, was found to react immediately at 0 °C with neat dimethylamine to form the expected[4] ring-opened adduct **6**. Moreover, reaction with 0.2 M ethyl glycinate in chloroform occurred to form an adduct **4**, with a half-time of roughly 13 h at 25 °C. By contrast, we observed no detectable reaction between neat piperidine, diethylamine, or 0.6 M ethyl glycinate and 2-methylchromone, **5b**. The latter did react slowly with neat ethylamine or dimethylamine, but the half-times, which were on the order of 0.5 h, were unsuitably long for desirable cleavage conditions. Not surprisingly, flavone (2-phenylchromone) failed to react with either ethylamine or dimethylamine, and 3,6-dimethylchromone (**7**), with a methyl α to the carbonyl that might be expected to reduce the relative stabilities of **4** and **6**, reacted slowly with neat

(1) Wünsch, E., "Syntheses von Peptiden", Bd15 Teil 1, "Houben-Weyl, Methoden der Organischen Chemie", E. Muller, Ed. G. Thieme, Stuttgart, 1974. "Protecting Groups in Organic Chemistry"; McOmie, J., Ed.; Plenum Press: New York, 1973.
(2) Kemp, D. S.; Hoyng, C. F. *Tetrahedron Lett.* **1975**, 4624.
(3) Stock, L. M.; Brown, H. C. *J. Am. Chem. Soc.* **1959**, *81*, 3323. Okamoto, Y.; Brown, H. C. *J. Org. Chem.* **1957**, *22*, 485.
(4) Zayorevskii, V.; Orlova, E.; Tsvetkova, I.; Vinokurov, V.; Troitskaya, V.; Rozenburg, S. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1971**, *7*, 675.
(5) For example, the pK_a values of H-Gly-OEt·HCl and H-Gly$_5$-OEt·HCl are 7.6 and 7.9, respectively. Perrin, D. D. "Dissocation Constants of Organic Bases in Aqueous Solution"; Butterworths: London, 1965; pp 384–385.

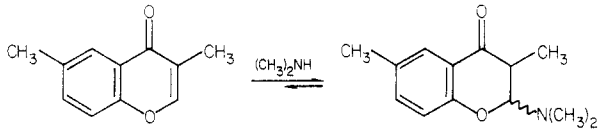
0022-3263/81/1946-4971$01.25/0

Table I. Analytical Data for Tcrom Esters[a]

acid	C	H	N	other
benzoic	62.07 (62.02)	3.18 (3.29)		F, 16.37 (16.52)
Z-L-Ala-OH	58.80 (58.75)	4.04 (4.22)	3.12 (3.15)	
Fmoc-L-Phe-OH	68.51 (68.56)	4.27 (4.32)	2.28 (2.37)	
Boc-L-Met-OH	53.07 (53.26)	5.05 (5.18)		S, 6.75 (7.03)

[a] Calculated (found).

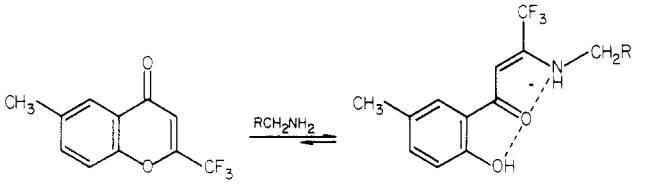
ethylamine to give an adduct of structure **4** and reversibly with dimethylamine to give a simple adduct **8**.



7 **8**

These experiments establish that the equilibrium **3** → **4** is highly sensitive to pyrone substituents and can be reversible. They also suggest that the optimal chromone should bear a 2-substituent that is bulkier than methyl (to further reduce reactivity toward secondary amines) and which induces a reactivity toward primary amines that lies between that of **5a** and **5b**. The 2-(trifluoromethyl)-6-methylchromone (**9**) readily prepared[6] from *p*-cresol by acetylation, Fries rearrangement, and Claisen condensation with ethyl trifluoroacetate, is an obvious choice.

When **9** was treated at 0 °C with neat dimethylamine or piperidine, addition to the pyrone α,β-double bond occurred, but no ring opening could be detected; moreover, reversion to **9** occurred if the piperidine adduct was allowed to remain overnight in chloroform. With the more hindered diethylamine, no addition was observed. However, formation of adduct **10** occurred upon mixing **9** with neat ethyl or propylamines. No reaction was detected between ethyl glycinate and **9** in chloroform solution within 2 days at 25 °C, but in acetonitrile a slow conversion to **10** occurred, with a half-life of 2 days at 0.2 M concentrations, and in DMF, a half-life of 12 h was seen.

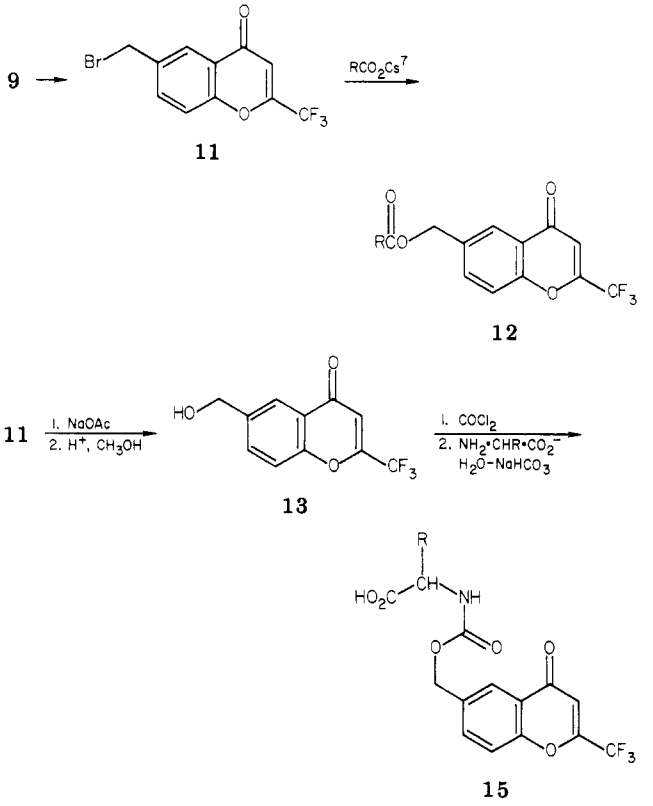


9 **10**

The amino function of glycine is the least hindered and one of the more basic of those belonging to the 20 common amino acids. With methyl alaninate (0.8 M) and **9** (0.4 M) in acetonitrile we observed no detectable addition or conversion to **10** in 6 days at 25 °C. In DMF under these conditions, at least 95% of **10** remained after 1 day. We attribute this striking inertness of **9** in the presence of an α-substitued amino acid ester to a destabilizing steric interaction between the trifluoromethyl group and the α-substituent of adduct **10**. This interaction must reverse the normal stability order of **9** and **10** at the amine concnetration of the study. It has the important practical consequence of allowing protective groups derived from **9** to be used in the presence of the concentrations of peptide-derived amines that are likely to be encountered in normal peptide synthesis.

(6) Whalley, W. B. J. *Chem. Soc.* **1951**, 3235.

Scheme I



11 **12** **13** **15**

Chromone-Derived Protective Groups

Bromination of **9** yields **11**, which is the natural precursor of protective groups of the benzyl ester (**12**) and benzyl urethane (**15**) classes, as shown in Scheme I. (Although the chloroformate (**14**) obtained from **13** can be isolated as a crystalline solid, the yields and purities of urethanes **15** obtained from stored chloroformate have been lower than those from freshly prepared material, and we have usually employed the latter.)

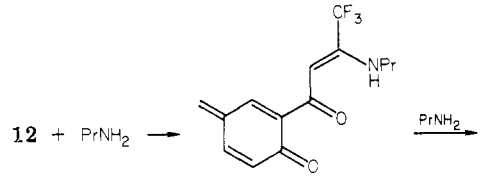
Preliminary studies of the stability and properties of these species were carried out on the benzoate ester **12** (R = Ph). When this species was treated with neat propylamine for 6 min at 25 °C and then with diethyl ether followed by evaporation, 91% of pure benzoic acid was recovered. When **12** (R = Ph) was dissolved in 0.5 M ethanolic hydrazine[8] at 25 °C for 5 min and then quenched with water and acid, workup gave a quantitative yield of crude and an 86% yield of pure benzoic acid. Similar results were obtained for the deprotection of **12** where R = Fmoc-L-Phe and **12** where R = Boc-L-Pro. Reaction times of 30–45 min appear to be necessary to effect cleavage of the urethane function of **15**. For example, the urethane **15** of L-Phe-OEt was dissolved in neat propylamine, and after 30 min at 25 °C the amine was evaporated and replaced by dichloromethane, which was again evaporated; 82% of the hydrochloride of H-L-Phe-OEt was isolated after acidic workup. The difference in rate of cleavage of esters **12** and urethanes **15** appears to result from a difference in cleavage rates of the respective enamides **10**, and not from a variation in ring-opening rates for the chromones.

Upon amine or hydrazine-induced fragmentation, **12** or **15** is expected to generate the quinonemethide **16** as a

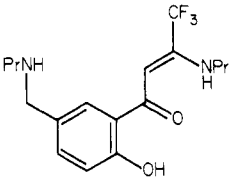
(7) Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.

(8) Under these conditions, the chromone-derived cleavage product is presumed to be the pyrazole.

transient intermediate. Completely effective trapping of this species is essential for trouble-free deblocking, since quinonemethides are capable of reacting with the nucleophilic groups of peptides, including the liberated α-amino chain terminus. Fortunately, the deblocking conditions consist of an excess of a good nucleophile; thus propylamine reacts with **16** to give **17**. This substance is lipophilic and difficult to extract from organic phases, even at low pH, and these properties of **17** make it easy to remove from deblocked peptides.



16



17

Although sensitive to unhindered bases such as hydroxide ion and partially decomposed by a 2-h exposure to tetramethylguanidine in acetonitrile, the 2-(trifluoromethyl)chromone function of **12** or **15** is stable to many acidic conditions. The benzoate ester **12** (R = Ph) is recovered unchanged from contact with dioxane saturated with hydrogen chloride for 30 min at 25 °C. No detectable change in ^{1}H NMR spectrum was observed for this substance after exposure to 4 M hydrogen bromide in acetic acid for 2 h at 25 °C. After 70 h under the latter conditions, 80% of cleavage to **11** was observed by ^{1}H NMR, which corresponds to roughly 1% cleavage during the normal 30-min reaction time used to remove the carbobenzoxyl group.

Chromones are unusually basic carbonyl derivatives (the pK_a of the conjugate acid of chromone itself is −2.0[9]), and protonation by hydrogen bromide may help to protect **12** from cleavage under acidic conditions. The stability to acids of esters **12** and urethanes **15** allows these groups to withstand the deblocking conditions that are useful to remove the Bpoc groups as well as the Boc, *tert*-butyl ester, and *tert*-butyl ether functions. The benzyl–oxygen bonds of **12** or **15** are cleaved by hydrogenolysis, and the chromone function appears to be reduced by zinc metal in acetic acid. These groups clearly do not withstand the commonly used reducing conditions for protective groups removal.[10] Although the Tcroc and Tcrom groups do not appear to be cleanly photolabile, we have noted discoloration without the appearance of NMR-detectable impurities in samples stored in clear glass vials and exposed to normal fluorescent lighting. Storage in amber bottles is therefore recommended.

Peptide Models

With some misgivings concerning the proliferation of acronyms for new blocking groups we propose the trivial names Tcroc (pronounced tee-crŏck) for the 2-(*trifluoro*-methyl)-6-*chr*omonylmethyleneoxycarbonyl function of **15** and Tcrom (pronounced tee-crŏm) for the 2-(*trifluoro*methyl)-6-*chr*omonyl*m*ethylene function of **12**. A first step toward the demonstration of the practicality of the Tcroc and Tcrom groups in peptide synthesis is given by the reaction sequences of Scheme II in which the removal of the *tert*-butyl ester function in the presence of the Tcroc group is demonstrated by the synthesis of Tcroc-L-Ala-Gly-OH, and the independent removal of both Boc and Tcrom groups is established by the three-step synthesis of Boc-protected methionine enkephalin.[11]

Scheme II

A. Removal of *tert*-Butyl Ester in the Presence of Tcroc

Tcroc-L-Ala-OH + H-Gly-OtBu $\xrightarrow[\text{DMF}]{\text{DCC–HOBt}^a}$ Tcroc-L-Ala-Gly-OtBu (53%)

Tcroc-L-Ala-OtBu $\xrightarrow[1\text{ h, }25\text{ °C}]{\text{Tfa}^a}$ Tcroc-L-Ala-Gly-OH (91%)

B. Removal of Boc in the Presence of Tcrom and Tcrom in the Presence of Boc

Boc-L-Met-OCs $\xrightarrow[\text{DMF, 5 h, 25 °C}]{\text{Tcrom-Br}}$ Boc-L-Met-Tcrom (62%)

Boc-L-Met-Tcrom $\xrightarrow[15\text{ min, }25\text{ °C}]{\text{HCl–dioxane}}$ C^-H_2-L-Met-Tcrom (86%)

Boc-Gly-L-Phe-OH + H-L-Met-Tcrom $\xrightarrow[\text{DMF}]{\text{DCC–HOBt}^a}$ Boc-Gly-L-Phe-L-Met-Tcrom (91%)

Boc-Gly-L-Phe-L-Met-Tcrom $\xrightarrow[20\text{ min, }25\text{ °C}]{\text{HCl–dioxane}}$ C^-H_2-Gly-L-Phe-L-Met-Tcrom (83%)

Boc-L-Tyr-Gly-L-Phe-L-Met-Tcrom $\xrightarrow[\text{DMF}]{\text{DCC–HOBt}^a}$ Boc-L-Tyr-Gly$_2$-L-Phe-L-Met-Tcrom (64%)

Boc-L-Tyr-Gly$_2$-L-Phe-L-Met-Tcrom $\xrightarrow[2\text{ min, }25\text{ °C}]{\text{PrNH}_2}$ Boc-L-Tyr-Gly$_2$-L-Phe-L-Met-OH (96%)

[a] DDC = dicyclohexylcarbodiimide, HOBt = 1-hydroxybenzotriazole,[12] Tfa = trifluoroacetic acid.

Noteworthy features of the Tcroc and Tcrom groups are their ultraviolet absorption maxima at 246 (10300) and 311 nm (5750), and the characteristic NMR chemical shift of the pyrone β-hydrogen at δ 6.78, which moves to δ 6.28 upon ring cleavage and conversion to enamides **10**. These spectroscopic properties can be used to monitor the purity of peptide intermediates and the completeness of deprotection steps.

In this study we have demonstrated that new chromone-bearing protective groups for carboxylic acids (Tcrom) and for amines (Tcroc) can be cleaved rapidly and completely by brief exposure to dilute ethanolic hydrazine or to unhindered primary amines, such as propylamine. Exposure to a weakly basic, α-branched primary amine such as methyl alaninate does not result in cleavage, and a similar inertness has been shown for secondary amines. The Tcrom ester and Tcroc urethane groups are particularly suited for use with Boc urethane and OtBu ester groups, since each class of group can be removed cleanly in the presence of the other, as demonstrated by a synthesis of N^α-Boc methionine enkephalin. The inertness of chromone groups to secondary amines and the successful conversion of Fmoc-L-Phe-Tcrom to Fmoc-L-Phe-OH suggest that Tcrom and Tcroc may be complementary to the Fmoc group, which is cleaved by brief treatment with

(9) Tolmachev, A. I.; Shulezhko, L. M.; Kisilenko, A. A. *J. Gen. Chem. USSR (Engl. Transl.)* **1965**, *35*, 1708.

(10) Stevenson, D.; Young, G. T. *J. Chem. Soc. C*, **1969**, 2389. Sheehan, J.; Guziec, F. *J. Am. Chem. Soc.* **1972**, *94*, 6561. Veber, D.; Brady, S.; Hirschmann, R. "Chemistry and Biology of Peptides"; Meienhofer, J., Ed.; Ann Arbor, MI, 1972; p 315.

(11) Hughes, J.; Smith, T.; Kosterlitz, H.; Fothergill, L.; Morgan, B.; Morris, H. *Nature (London)* **1975**, *258*, 577.

(12) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788, 2024, 2034.

secondary amines (10% piperidine to DMF).[13]

This study is a first step toward establishing the practicality and reliability of the Tcrom and Tcroc groups for peptide synthesis. More rigorous demonstrations of the utility of these groups, including a synthesis of the peptide hormone somatostatin, are in progress and will be reported subsequently.

Experimental Section

Melting points were taken with a Thomas-Hoover Unimelt apparatus. Infrared spectra were recorded on Perkin-Elmer 567 or 283B spectrophotometers. Proton NMR spectra were measured with Varian T-60 or Hitachi Perkin-Elmer R24B spectrometers, and mass spectra were obtained with a Varian MAT-44 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. HPLC analyses were conducted with a Waters Associates liquid chromatograph with a UV monitor and a Model 660 solvent programmer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Thin-layer chromatography was conducted on Merck F-254 silica-coated glass plates. Unless otherwise noted, solvents were reagent grade and used without purification, evaporations were conducted at water aspirator pressure with a Büchi rotary evaporator, and solvent extracts were dried over anhydrous sodium sulfate.

6-Methyl-2-(trifluoromethyl)chromone (9). A modification of the procedure of Whalley[5] was followed: **2-Hydroxy-5-methylacetophenone.** To a solution of *p*-cresol (600 g, 5.5 mol) in acetic anhydride (1500 mL) is added pyridine (45 mL). After 12 h of stirring at 25 °C, the volatiles are evaported (a Büchi rotary evaporator containing a liquid-nitrogen-cooled cold finger is used to remove final traces). The resulting yellow oil is taken up in diethyl ether (1 L), washed with 10% aqueous sodium bicarbonate (2 × 150 mL) and once with brine, and dried. Evaporation in vacuo gives *p*-cresol acetate as an oil (734 g, 88%). To this crude ester is added anhydrous aluminum chloride (750 g) in portions. The dark, viscous mixture is heated 1 h at 130 °C, cooled to 25 °C, treated with ice (300 g), allowed to stand for 1 h, diluted with dichloromethane (500 mL), and stirred overnight. The organic phase is separated, dried, and evaporated to give a purple oil, which is distilled, bp 83–87 °C at 0.5 mm (720 g, 98%). Upon seeding, the oil solidifies.

To a solution obtained from sodium (50 g) in ethanol is added the freshly distilled 2-hydroxy-5-methylacetophenone (150 g, 1 mol) with vigorous stirring. As soon as the solution sets to a solid mass, ethyl trifluoroacetate (300 g, 2.1 mol) is added, and the mixture is heated to reflux for 5 h and then cooled and poured onto ice (1 kg) and 0.5 M citric acid (1.5 L). The resulting precipitate of hemiketal is collected and recrystallized from methanol–water to give white prisms: mp 155–160 °C (lit.[5] mp 157 °C); 171 g, 70%; ^{1}H NMR ($CDCl_3$–CD_3CN) δ 2.40 (s, 3 H), 3.08 (br d, 2 H), 5.75 (br s, 1 H), 6.9–7.7 (m, 3 H).

The above hemiketal is dissolved in ethanol (1.2 L), treated with 12 M hydrochloric acid (240 mL); the mixture is refluxed for 30 min, cooled, and evaporated (ethanol only). The resulting mass is triturated with dichloromethane (200 mL), and the resulting extract is washed with water, dried, and evaporated to give an oil which is taken up in methanol (100 mL) and cooled to −20 °C. The chromone crystallizes as large prisms: mp 48–51 °C (lit.[5] mp 53 °C); 95 g, 60%; IR ($CHCl_3$) 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3 H), 6.72 (s, 1 H), 7.0–8.1 (m, 3 H); UV max (EtOH) 246 nm (ε 10 300), 311 (5750).

6-(Bromomethyl)-2-(trifluoromethyl)chromone (11). A mixture of **9** (14.6 g, 64 mmol), *N*-bromosuccinimide (12.1 g, 67 mmol), azobis(isobutyronitrile) (50 mg), and carbon tetrachloride (450 mL) is irradiated with a 250-W incandescent bulb and warmed with a heat gun. Reaction is completed by heating to reflux for 1.5 h, and the suspension is cooled, filtered, and evaporated. Cyclohexane (15 mL) is added to the resulting yellow oil, and the product is obtained as needles, mp 85–90 °C (9.6 g, 49%). Recrystallization gave the following: mp 97–98 °C; IR ($CHCl_3$) 1663 cm^{-1}; mass spectrum *m/e* 306 (M^+), 308; ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2 H), 6.80 (s, 1 H), 7.4–8.3 (m, 3 H).

Anal. Calcd for $C_{11}H_6O_2F_3Br$: C, 43.0; H, 2.0; Br, 26.0. Found: C. 43.0; H, 1.8; Br, 26.2.

6-(Hydroxymethyl)-2-(trifluoromethyl)chromone (13) and Chloroformate (14). A solution of bromide 11 (5 g, 16 mmol) and sodium acetate trihydrate (8 g) in acetic acid (40 mL) is brought to reflux for 6 h, cooled to 25 °C, diluted with water, and filtered. The resulting precipitate is dissolved in methanol, and the solution is then treated with 12 M hydrochloric acid (3 mL) and stirred for 6 h at 25 °C. Evaporation at aspirator pressure is followed by distillation in a Kugelrohr at 120 °C (0.5 mm); the crystalline distillate is recrystallized from ethyl acetate–petroleum ether to give alcohol 13: mp 94–95 °C, 1.6 g, 41%; IR ($CHCl_3$) 3600, 3450, 1662 cm^{-1}; mass spectrum, *m/e* 244 (M^+); ^{1}H NMR ($CDCl_3$) δ 3.9 (br s, 1 H), 4.78 (s, 2 H), 6.6 (s, 1 H), 7.3–8.0 (m, 3 H).

For conversion to the chloroformate **14**, alcohol **13** is dissolved in dichloromethane containing 3–5 equiv of phosgene. After 5 h at 25 °C, evaporation gives a colorless oil which solidifies on standing: mp 50–54 °C; ^{1}H NMR ($CDCl_3$) δ 5.23 (s, 2 H), 6.51 (s, 1 H), 7.3–8.1 (m, 3 H).

Formation of Tcrom Esters (12): Z-L-Leu-OTcrom. A mixture of bromide 11 (200 mg, 0.65 mmol) and cesium Z-L-leucinate[7] (260 mg, 0.65 mmol) in 2 mL of DMF was stirred at 25 °C for 4 h, the solvent was then evaporated, and the residue was taken up in ether, washed with water, dried, and evaporated. The resulting solid was recrystallized from ethanol to yield Z-L-Leu-OTcrom as fine needles: mp 94–97 °C; 170 mg, 53%; $[\alpha]^{21.5}_D$ −9.5° (*c* 3.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 6 H), 1.7 (m, 3 H), 4.6 (m, 1 H), 5.20 (s, 2 H), 5.35 (s, 2 H), 5.5 (m, 1 H), 6,80 (s, 1 H), 7.40 (s, 5 H), 7.5–8.3 (m, 3 H).

Anal. Calcd for $C_{25}H_{24}NO_6F_3$: C, 61.09; H, 4.92; N, 2.85. Found: C. 60.94; H, 4.97; N, 2.83.

By this procedure were also prepared the Tcrom esters of the following acids: benzoic acid, mp 91–93 °C (68%); Z-L-Ala-OH, amorphous (85%); Boc-L-Pro-OH, oil (83%); Fmoc-L-Phe-OH, mp 102–107 °C (89%); Boc-L-Met-OH, mp 98–100 °C (62%).

Formation of Tcroc Urethanes (15): Tcroc-L-Ala-OH. To a stirred solution of L-alanine (400 mg, 4.4 mmol) in 5 mL of water containing sodium bicarbonate (300 mg) was added chloroformate 14 (0.7 g, 2.2 mmol) in 2 mL of dioxane. After 2 h at 25 °C, the solution was extracted with dichloromethane, acidified to pH 3 with citric acid, and extracted with three portions of dichloromethane. The pooled extracts were dried and evaporated, and the residue was recrystallized at −20 °C from chloroform–petroleum ether to give fine needles: mp 127–128 °C; 0.50 g, 63%; ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3 H), 4.40 (m, 1 H), 5.20 (s, 2 H), 6.06 (br d, 1 H), 6.72 (s, 1 H), 7.4–8.1 (m, 3 H), 9.6 (s, 1 H).

Anal. Calcd for $C_{15}H_{12}NO_6F_3$: C, 50.14; H, 3.36; N, 3.89; F, 15.87. Found: C, 50.14; H, 3.50; N, 3.73; F, 15.80.

Tcroc-L-Phe-Oet was prepared by reaction of chloroformate 14 with 2 equiv of H-L-Pheh-OEt in dichloromethane: mp 102–107 °C; $[\alpha]^{23}_D$ +31.3 (*c* 1.3, $CHCl_3$).

Anal. Calcd for $C_{23}H_{20}NO_6F_3$: C, 59.61; H, 4.34; N, 3.02. Found: C, 59.50; H, 4.41; N, 3.02.

Tcrom Ester of *tert*-Butoxycarbonylglycyl-L-phenylalaninyl-L-methionine. Dioxane (10 mL) containing 0.5 mL of anisole was saturated with hydrogen chloride, and Boc-L-Met-OTcrom (0.70 g, 1.5 mmol) was added. After 15 min at 25 °C, the solvent was evaporated, and the resulting oil was triturated under ether. The resulting white powder, H-L-OTcrom-HCl, was washed with ether and dried in a desiccator (0.52 g, 86%).

A soluition of this hydrochloride salt (0.50 g, 1.2 mmol) and Boc-Gly-L-Phe-OH (0.39 g, 1.2 mmol) in freshly distilled DMF (10 mL) was cooled to 0 °C and treated with hydroxybenzotriazole (165 mg), triethylamine (170 μL), and dicyclohexylcarbodiimide (300 mg). The stirred reaction was continued for 2 h at 0 °C and for 13 h at 25 °C. Water and ethyl acetate were then added, the mixture was filtered, and the organic phase was washed with water (2 × 20 mL), 0.5 M citric acid (2 × 20 mL), 5% sodium bicarbonate (2 × 20 mL), and water (20 mL). Drying and evaporation was followed by solution in ethyl acetate, filtration, and evaporation until the urea was no longer collected. drying by azeotropic evaporation of acetonitrile followed by trituration under ether yielded 0.75 g (91%) of a solid showing one major component with

(13) Atherton, E.; Fox, H.; Harkiss, D.; Logan, C. J.; Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1978**, 537.

traces of two impurities by TLC ($CHCl_3$–CH_3OH, 9:1, on EtOAc). Precipitation from methanol with water gave a solid, mp 95–99 °C, which was homogeneous by TLC: $[\alpha]^{20}_D$ −14.7° (c 1.9, $CHCl_3$).

Anal. Calcd for $C_{32}H_{36}N_3O_8SF_3$: C, 56.54; H, 5.33; N, 6.18. Found: C, 56.38; H, 5.43; N, 6.16.

Tcrom Ester of *tert*-Butoxycarbonyl-L-tyrosylglycylglycyl-L-phenylalaninyl-L-methionine. The tripeptide prepared above (210 mg, 0.30 mmol) was dissolved in dioxane (2 mL) and anisole (0.5 mL) which had been saturated with hydrogen chloride. After 20 min at 25 °C the solvent was evaporated, and the residue was triturated with ether to give the hydrochloride salt of H-Gly-L-Phe-L-Met-OTcrom as a white powder (159 mg, 83%). This was dissolved in 5 mL of freshly distilled DMF containing *tert*-butoxycarbonyl-L-tyrosylglycine (88 mg, 0.26 mmol). The solution was cooled to 0 °C and treated with hydroxybenzotriazole (40 mg), triethylamine (36 μL), and dicyclohexylcarbodiimide (65 mg). After 2 h at 0 °C and 15 h at 25 °C, water and ethyl acetate were added, the slurry was filtered, and the filtrate was evaporated, ultimately at 0.1 mm. The resulting residue was taken up in ethyl acetate, washed with water, 0.5 M citric acid, 5% sodium bicarbonate, and water, and then dried and evaporated. The resulting oil was dried by repeated evaporation of acetonitrile, and the residue was triturated with ethyl acetate to give a white powder (143 mg, 64%) which was homogeneous by TLC ($CHCl_3$–CH_3OH, 9:1): $[\alpha]^{20}_D$ −11.5° (c 1.7, CH_3OH–$CHCl_3$, 3:1, v/v).

Anal. Calcd for $C_{43}H_{48}H_5O_{11}SF_3$: C, 57.39; H, 5.37; N, 7.78. Found: C, 57.29; H, 5.50; N, 7.68.

N^{α}-Boc Methionine Enkephalin. The above Tcrom ester of N^{α}-Boc methione enkephalin (28 mg, 0.03 mmol) was dissolved in 2 mL of propylamine. After 2 min at 25 °C the amine was evaporated, and the residue was triturated with ether. The bright yellow suspension was filtered, and the collected white solid was dissolved in 5 mL of water. The soluition was filtered, acidified to pH 2–3 with citric acid, and extracted with ethyl acetate, which was then washed with water, dried, and evaporated to yield 20.3 mg (96%) of a white solid, mp 120–135 °C, which was homogeneous by TLC in two solvent systems ($CHCl_3$–CH_3OH–HOAc, 9:1:1, and *n*-butanol–ACOH–H_2O, 7:2:1) and which showed an HPLC trace identical with that of a standard sample (CH_3OH–H_2O, 2:3, flow rate 1 mL/min, μ-Bondapak, C_{18} reverse phase).

Anal. Calcd for $C_{32}H_{43}N_5O_9S$ (for the standard sample): C, 57.04; H, 6.43; N, 10.39; S, 4.75. Found: C, 56.89; H, 6.50; N, 10.36; S, 4.71.

Tcroc-L-alaninylglycine *tert*-Butyl Ester and Tcroc-L-alaninylglycine. A solution of Tcroc-L-Ala-OH (320 mg, 0.90 mmol) and *tert*-butyl glycinate phosphite salt (227 mg, 1.0 mmol) in 3 mL of DMF was cooled to 0 °C and treated with hydroxybenzotriazole (140 mg), triethylamine (130 μL), and dicyclohexylcarbodiimide (220 mg). After 4 h at 0 °C and 12 h at 25 °C, the mixture was worked up as described for the preparation of Boc-Gly-L-Phe-L-Met-OTcrom. The resulting powder was triturated with ether to give 225 mg (53%) of product that was homogeneous by HPLC analysis (CH_3OH–H_2O, 4:1, containing 0.4% HOAc; 1 mL/min, μ-Bondapak C_{18}).

Anal. Calcd for $C_{21}H_{23}N_2O_7F_3$: C, 53.39; H, 4.90; N, 5.93; F, 12.05. Found: C, 53.54; H, 5.04; N, 5.91; F, 11.94.

After 1 h at 25 °C a solution of the above ester (68.5 mg, 0.145 mmol) in 1.5 mL of trifluoroacetic acid was evaporated. Methanol was added (2 × 5 mL) and evaporated to yield a tan solid, 60.6 mg (100%). Recrystallization from acetonitrile gave 54.9 mg (91%), mp 141–143 °C, of Tcroc-L-Ala-Gly-OH.

Anal. Calcd for $C_{17}H_{15}N_2O_7F_3$: C, 49.05; H, 3.63; N, 6.73; F, 13.69. Found: C, 48.83; H, 3.76; N, 6.59; F, 13.58.

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Crystal and Molecular Structure of Diphenylmethane

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The determination of the X-ray structure of diphenylmethane at −70 °C is the first of its kind for a molecule of the type Ph_2ZH_2, where Z is an element of group 4A. Crystals are monoclinic, space group $P2_1/c$, a = 8.875 (11) Å, b = 6.220 (12) Å, c = 20.232 (19) Å, β = 119.89 (9)°. The structure is of the helical type, with ring twist angles of 63.9° and 71.1°. The central C–C–C bond angle of 112.5° is significantly smaller than any such angle previously reported for a ring-substituted diphenylmethane. Empirical force field (EFF) and molecular orbital (EHT, MNDO) calculations indicate a C_{2v} (gable) ground state for the isolated molecule, which is only ca. 0.5 kcal mol^{-1} lower in energy than the helical conformation.

Structural studies of molecules containing at least two substituted or unsubstituted benzene rings attached to a common atomic center are legion: counting X-ray structures alone, 12% of the studies reported in the Cambridge crystallographic database deal with molecules of this description.[2] It is therefore somewhat surprising that not a single X-ray structure has been reported to date for an unsubstituted molecule of the type Ph_2ZH_2 (where Ph = C_6H_5 and Z is an element of group 4A),[4] despite a prodigious amount of work devoted to the conformational analysis of these (and related) compounds. The present study was undertaken in part to remedy this situation.

Diphenylmethane (DPM) was chosen as the target molecule for this investigation since DPM is the parent compound to which all others of the type Ph_2ZH_2, Ph_2ZH, and Ph_2Z may be related as heteroatom derivatives. Also, because DPM is a hydrocarbon, its conformational energies are reliably calculated by the empirical force field (EFF) method.[5] Finally, in contrast to compounds of the types

(1) (a) The University, Dundee. (b) Princeton University.

(2) This statistic is based on a search covering the literature from 1935[3] and updated to January, 1981. The total number of X-ray structures in this file is 27 551, and the number of hits for the $(C_6^{ar})_2X$ fragment is 3265.

(3) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr., Sect. B* **1979**, *35*, 2331. Wilson, S. R.; Huffman, J. C. *J. Org. Chem.* **1980**, *45*, 560.

(4) To the best of our knowledge, the only X-ray structure reported for a representative of type Ph_2ZH (Z = group 5A element) is that of the 1:1 benzophenone–diphenylamine complex: Brassy, C.; Mornon, J.-P. *Compt. Rend. C* **1972**, *274*, 1728. For type Ph_2Z (Z = group 6A element), there is only one allusion to an X-ray study of diphenyl ether, which is consistent with a helical structure ($\phi_A = \phi_B$ = 17.5°): Katayama, M., unpublished work cited in Higasi, K., *Monogr. Ser. Res. Inst. Appl. Electr., Hokkaido Univ.* **1965**, *13*, 29.

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