Solid-Phase Synthesis of Peptidyl Trifluoromethyl Ketones

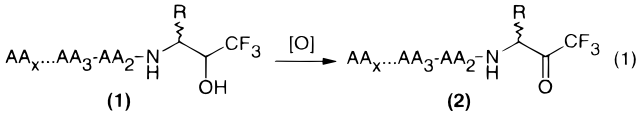
Marc-André Poupart,* Gulrez Fazal, Sylvie Goulet, and Ly Thy Mar

Boehringer Ingelheim (Canada) Ltd., Bio-Méga Research Division, 2100 Cunard Street, Laval, Québec, Canada H7S 2G5

Received July 30, 1998 (Revised Manuscript Received December 3, 1998)

Serine proteases constitute attractive biological targets due to their implication in a number of important biological events.[1] A wide range of substances have been found to interfere with the catalytic machinery of serine proteases. Among these, substrate-based trifluoromethyl ketones constitute a particularly well documented class of serine protease inhibitors, which have proven effective against elastase,[2] chymotrypsin,[3] and CMV protease.[4]

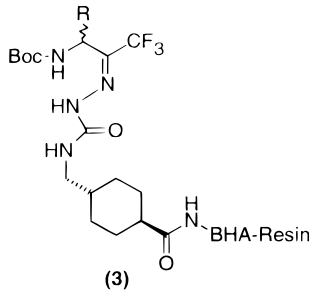
The synthesis of peptidyl trifluoromethyl ketones (**2**) is typically performed in solution, by preparing a precursor trifluoromethyl alcohol (**1**) and subjecting it to a final oxidation step (eq 1).[5] While this approach is well suited



(1) (2) (1)

in most cases, the final oxidation step may limit the choice of functional groups to be incorporated in the inhibitor.

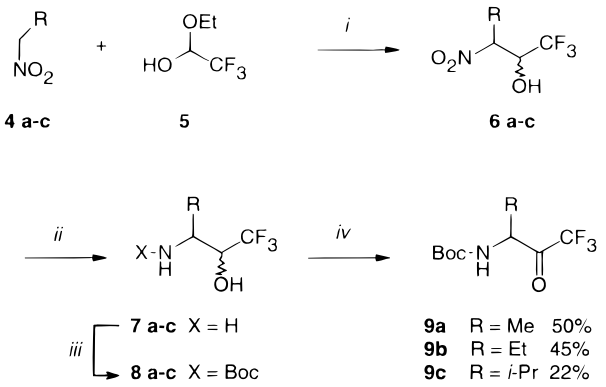
To take advantage of recent advances in robotic technology and combinatorial chemistry,[6] we sought to develop a solid-phase process for the synthesis of peptidyl trifluoromethyl ketone inhibitors. Our goal was to develop a method that would give direct access to the trifluoromethyl ketone functionality, thus avoiding the need to perform a final oxidation step. To this end, we considered using a semicarbazone linkage (**3**) to serve



(3)

Figure 1.

Scheme 1[a]



[a] (i) K_2CO_3 (cat.); (ii) Ra−Ni, H_2 (40 psi)/MeOH; (iii) Boc_2O, $NaHCO_3$/THF, H_2O; (iv) oxalyl chloride, DMSO/CH_2Cl_2, then Et_3N, −78 to 0 °C.

both as a reversible protecting group for the ketone and also as an anchoring group to the polymeric support[7,8] (Figure 1).

The various protected trifluoromethyl ketone synthons **9a**−**c** were synthesized in four steps as illustrated in Scheme 1. Thus, a Henry reaction[5] between a suitable nitroalkane **4a**−**c** and trifluoroacetaldehyde ethyl hemiacetal **5** afforded the crude nitro alcohols **6a**−**c**. These were hydrogenated over Raney-nickel and the resulting amino alcohols **7a**−**c** converted to the *N*-Boc derivatives **8a**−**c**. Following a Swern oxidation, the racemic *N*-Boc trifluoromethyl ketones **9a**−**c** were isolated in 22−50% overall yields.

With the necessary trifluoromethyl ketones in hand, it remained to generate the desired semicarbazone moiety

(1) Review articles: (a) Demuth, H.-U. *J. Enzyme Inhibition* **1990**, *3*, 249. (b) Mehdi, S. *Bioorg. Chem.* **1993**, *21*, 249. (c) Hörl, W. H. Inhibition of Proteinases. In *Design of Enzyme Inhibitors as Drugs*; Sandler, M., Smith, H. J., Eds.; Oxford University Press: Oxford, 1989; Chapter 18.

(2) Edwards, P. D.; Bernstein, P. R. *Med. Chem. Rev.* **1994**, *14*, 127 and references therein.

(3) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760.

(4) Ogilvie, W.; Bailey, M.; Poupart, M.-A.; Abraham, A.; Bhavsar, A.; Bonneau, P.; Bordeleau, J.; Bousquet, Y.; Chabot, C.; Duceppe, J.-S.; Fazal, G.; Goulet, S.; Grand-Maître, C.; Guse, I.; Halmos, T.; Lavallée, P.; Leach, M.; Malenfant, E.; O'Meara, J.; Plante, R.; Plouffe, C.; Poirier, M.; Soucy, F.; Yoakim, C.; Déziel, R. *J. Med. Chem.* **1997**, *40*, 4113.

(5) (a) Reference 3, supplementary material. (b) Imperiali, B.; Abeles, R. H. *Tetrahedron Lett.* **1986**, *27*, 135. (c) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *J. Med. Chem.* **1990**, *33*, 394. (d) Warner, P.; Green, R. C.; Gomes, B.; Strimpler, A. M. *J. Med. Chem.* **1994**, *37*, 3090. (e) Skiles, J. W., Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Mauldin, J. V.; Mui, P. W.; Jacober, S.; Chow, G.; Matteo, M.; Skoog, M.; Weldon, S. M.; Possanza, G.; Keirns, J.; Letts, G.; Rosenthal, A. S. *J. Med. Chem.* **1992**, *35*, 641. (f) Bergeson, S. H.; Schwartz, J. A.; Stein, M. M.; Wildonger, R. A.; Edwards, P. D.; Shaw, A.; Trainor, D. A.; Wolanin, D. J. European Patent Application 0 189 305, 1986.

(6) Reviews: (a) Chaiken, I. M.; Janda, K. D. *Molecular Diversity and Combinatorial Chemistry Libraries and Drug Discovery*; American Chemical Society: Washington, DC, 1996. (b) Abelson, J. N. *Methods in Enzymology, Combinatorial Chemistry*; Academic Press: New York, 1996; Vol. 267. (c) DeWitt, S. H.; Schroeder, M. C.; Stankovic, C. J.; Strode, J. E.; Czarnik, A. W. *Drug Dev. Res.* **1994**, *33*, 116. (d) DeWitt, S. H.; Czarnik, A. W. *Acc. Chem. Res.* **1996**, *29*, 114. (e) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135. (f) Eichler, J.; Appel, J. R.; Blondelle, S. E.; Dooley, C. T.; Dörner, B.; Ostresh, J. M.; Pérez-Payá, E.; Pinilla, C.; Houghten, R. A. *Med. Res. Rev.* **1995**, *15*, 481. (g) Thompson, L. A.; Ellman; J. A. *Chem. Rev.* **1996**, *96*, 555. (h) *Chem. Rev.* **1997**, *97*, March/April issue. (i) Wilson, S. R.; Czarnik, A. W. *Combinatorial Chemistry; Synthesis and Applications*; John Wiley and Sons: New York, 1997.

(7) Presented at the 213th National Meeting of the American Chemical Society, San Francisco, CA, April 13−17, 1997; Paper ORGN63.

(8) A similar solid-phase process for the preparation of peptidyl aldehydes has been reported: Murphy, A. M.; Dagnino, R., Jr.; Vallar, P. L.; Trippe, A. J.; Sherman, S. L.; Lumpkin, R. H.; Tamura, S. Y.; Webb, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 3156.

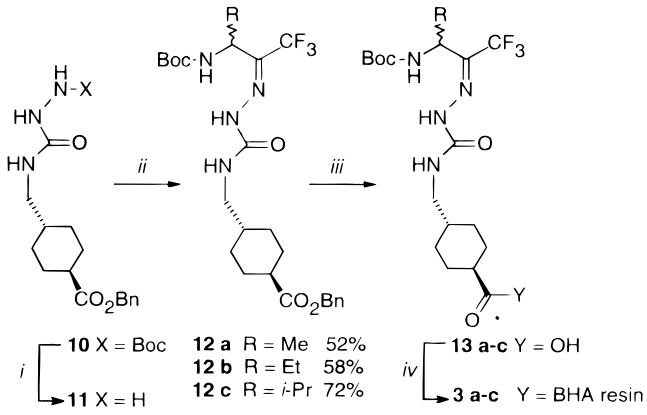
10.1021/jo9815204 CCC: $18.00
Published on Web 01/27/1999

Table 1. Peptidyl Trifluoromethyl Ketones Prepared

no.	compd	sequence[a]	synthetic protocol[b]	no. of cleavage cycles[b]	overall yield,[c] %
1	**14**	Ac−Ser-Tyr-Val-Lys-Ala-CF_3	2	3	28
2	**15**	Ac−Asn-Asp(OBn)-Leu-Ala-CF_3	3	2	40
3	**16**	Ph-C(O)-Glu-Tyr-Gly-Leu-Ala-CF_3	2	2	36
4	**17**	Ac−Phe-Leu-His-Thr-Ala-CF_3	2	3	19[d]
5	**19**	Ac-Gly-Val-Val-Asn-Ala-CF_3	2	2	30
6	**20**	Ac−Asp-Glu-Met-Glu-Glu-Abu-CF_3	2	2	36
7	**21**	Boc-Gly-Phe-Leu-Abu-CF_3	4	2	23
8	**22**	Boc-Val-Ser(Bn)-Gly-Asp(OBn)-Abu-CF_3	4	2	29
9	**23**	Asp(OBn)-Ala-Pro-Abu-CF_3	3	4	40
10	**24**	Boc-Ala-Ala-Pro-Val-CF_3	1	2	28
11	**25**	Ph-CH_2−C(O)-Tyr-Ala-Lys-Val-CF_3	2	4	18
12	**26**	Ac-Leu-Gly-Asp(OBn)-Ala-Val-CF_3	3	5	15
13	**27**	Ac-Gly-Ser(Bn)-Leu-Asp(OBn)-Val-CF_3	3	5	16
14	**28a,b**[e]	Ac−Phe-Val-Pro-Val-CF_3	1	2	16

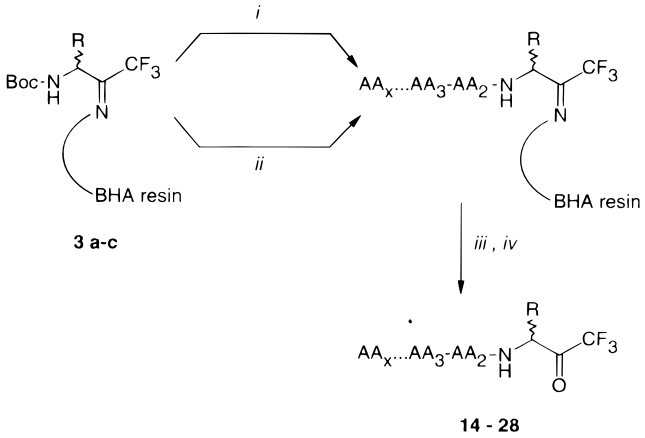
[a] 1:1 mixture at the trifluoromethyl ketone side chain unless otherwise stated. [b] See Experimental Section for details. [c] Overall yields are for purified, lyophilized compounds. [d] 6% of the protected peptide Thr(*t*-Bu) derivative **18** also isolated. [e] Both diastereomers separated by semipreparative HPLC.

Scheme 2[a]



[a] (i) 4 M HCl/dioxane, then aqueous K_2CO_3 (81%); (ii) **9a**−**c**, *p*-TsOH (cat.), toluene reflux; (iii) Pd/C, H_2 (40 psi)/MeOH−EtOAc; (iv) BHA resin, TBTU, HOBt, DIPEA/DMSO, NMP.

Scheme 3[a]



[a](i) (a) 45% TFA/CH_2Cl_2; (b) 5% DIPEA/CH_2Cl_2; (c) Boc-AA, coupling agent, HOBt/DMF; (d) repeat from step (a) as needed; (ii) (a) 45% TFA/CH_2Cl_2; (b) 5% DIPEA/CH_2Cl_2; (c) Fmoc-AA, coupling agent, HOBt/DMF; (d) 25% piperidine/DMF; (e) repeat from step (c) as needed; (iii) 75% TFA/CH_2Cl_2 (side chains deprotection if needed); (iv) AcOH, aqueous HCl/THF/65 °C, 4 h (cleavage from the resin).

12a−**c** and anchor it onto a BHA resin (see Scheme 2). To this end, the *N*-Boc-protected semicarbazide **10**[8] was deprotected and neutralized to give semicarbazide **11**. This compound was then subjected to an acid-catalyzed condensation with trifluoromethyl ketones **9a**−**c** in refluxing toluene to give predominantly the *E* semicarbazones **12a**−**c**[9] in moderate yields. The selective hydrogenolysis of the benzyl ester proceeded in high yield to give the corresponding acids **13a**−**c**. Those were coupled through their corresponding HOBt esters to a polystyrene BHA resin to afford the desired resins **3a**−**c**.

The solid-phase synthesis using resins **3a**−**c** was accomplished by standard protocols.[10] Since semicarbazones are resistant to both anhydrous acidic and mildly basic conditions, both *N*-Boc- and *N*-Fmoc-protected amino acids could be used at *any* position of the peptide sequence. This versatility proved to be of great value, as it allowed for an increased diversity of functional group in the final inhibitors. The coupling of amino acids was achieved through the corresponding HOBt esters as shown in Scheme 3.

At the completion of the synthesis, the acid-sensitive side chain protecting groups were removed by treatment with 75% TFA in CH_2Cl_2 for 3 h. The peptidyl trifluoromethyl ketone was released from the polymer support by refluxing the dried resin in a THF solution containing aqueous HCl and acetic acid at 65 °C (pH ∼ 2). After 4 h, the resin was filtered and the mother liquors were analyzed by HPLC to ascertain the extent of cleavage. In some cases it was necessary to repeat this sequence several times in order to liberate the maximum amount of compound (see Table 1 for specific cases).[11] In general, the rate of cleavage from the resin was slightly slower for valine-derived trifluoromethyl ketones **24**−**28** (Table 1) than for their alanine (**14**−**19**) or ethyl glycine (**20**−**23**) counterparts. When a basic residue was present in the peptide sequence, a slightly higher concentration of HCl was used and extra cleavage cycles were required in order to ensure maximum yields. We have found the

(9) In a separate experiment, when the condensation was done in refluxing benzene, a nearly 1:1 ratio of isomers was obtained. The isomers were separated and characterized separately. The *E*/*Z* assignment is based on ^{1}H NMR NOE experiments.

(10) (a) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis; A Practical Approach*; IRL Press: Oxford, 1989. (b) Grant, G. A. *Synthetic Peptides; A User's Guide*; W. H. Freeman: New York, 1992. (c) Atherton, E.; Sheppard, R. C. The Fluorenylmethoxycarbonyl Amino Protecting Group. In *The Peptides; Analysis, Synthesis, Biology*; Udenfriend, S., Meienhofer, J., Eds.; Special Methods in Peptide Synthesis Part C.; Academic Press: New York, 1987; Vol. 9, pp 1−38.

(11) Decomposition of the final product was observed following prolonged heating. Therefore, we found it more efficient to keep the reaction time to 4 h and repeat the hydrolytic treatment more than once.

addition of formaldehyde[8] (to trap the liberated semicarbazide) to be superfluous. This additive complicated the purification of the desired products (particularly for sequences containing a free amino group) and did not provide any significant benefit, as reflected by the overall yield of compound.

As reported in Table 1, several peptidyl trifluoromethyl ketones incorporating a wide variety of functional elements were synthesized by this new methodology. The crude products were typically 60−80% homogeneous as determined by analytical RP-HPLC and could easily be purified by semipreparative RP-HPLC to a homogeneity generally exceeding 95%. The overall yields of the purified material, which range from 15% to 40%, are sufficient to support a lead optimization effort.[4] Some of the lower yields may be a reflection of the individual degree of difficulty of purifying the desired compound to an acceptable homogeneity. In addition, incomplete cleavage from the resin (especially in cases of valine-derived trifluoromethyl ketone) may contribute to lower overall yields (for example, see entries 10−14, Table 1). Since the starting trifluoromethyl ketone fragments **9a**−**c** were racemic, the final compounds were isolated as a 1:1 mixture of diastereomers at the TFMK α-center.[12]

In most cases, the removal of all acid-sensitive protecting groups could be effected by pretreatment with 75% TFA prior to releasing the compound from the solid support. In a few examples, however, the deprotection of *O*-*tert*-butyl ethers was incomplete (Table 1, entry 4). During the final cleavage, no interference was observed from unprotected nucleophilic or oxidizable side chains such as those present in serine, methionine, tyrosine, histidine, lysine, or aspartic acid.[13] The cleavage conditions were mild enough to be compatible with various acid-sensitive protecting groups such as *N*-Boc (entries 7, 8, and 10) and *O*-Bn ester (entries 2, 8, 9, 12, and 13). It was also observed that under the cleavage conditions methyl esters were hydrolyzed to an extent of 50% (data not shown).

Conclusion

The solid-phase preparation of peptidyl trifluoromethyl ketones using a semicarbazone linker as anchoring point has been described. The chemistry is compatible with both *N*-Boc- and *N*-Fmoc-protected amino acids and affords the desired compound in 15−40% overall yield. The versatility of this approach was exemplified by the synthesis of more than 100 peptidyl trifluoromethyl ketones as HCMV protease inhibitors.[4] By its generality, this methodology is well suited for application in rapid lead optimization as well as for the generation of libraries directed toward the identification of novel serine protease inhibitors containing a trifluoromethyl ketone moiety.

Experimental Section[14]

3-Amino-1,1,1-trifluorobutan-2-ol (7a).[15] A solution of nitroethane **4a** (0.20 mol, 14 mL), trifluoroacetaldehyde ethyl hemiacetal 90% (0.20 mol, 26 mL), and anhydrous K_2CO_3 (4 mol %, 0.008 mol, 1.10 g) was heated at 50 °C behind a safety shield for 6 h and at room temperature for 40 h. The mixture was diluted with diethyl ether and then successively washed with 5% citric acid, a saturated aqueous solution of $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and most of the ether was evaporated under reduced pressure to give a yellow oil. The oil was dissolved in absolute ethanol (150 mL) and then hydrogenated at 40 psi over 1.5 g of ethanol-washed Raney nickel (from 3 g of a 50% aqueous suspension) until no more hydrogen was taken up. The solution was degassed, the catalyst was filtered over Celite, and most of the ethanol was evaporated under reduced pressure. The residue was diluted in 1:1 ether−hexane (200 mL) and left at −20 °C for 48 h. The greenish precipitate which formed was collected and washed with hexane (one diastereomer, 6.59 g, 23% overall). Greenish precipitate: mp 67−69 °C; ^{1}H NMR (DMSO-d_6) δ 3.81−3.74 (m, 1 H), 3.70−2.40 (broad s, 2 H), 2.98 (broad t, J = 5.2 Hz, 1 H), 1.02 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 126.4 (q, J = 284 Hz), 73.6 (q, J = 26.6 Hz), 47.1, 18.7; FT-IR (KBr) ν 3070, 2980 cm^{-1}; HR-MS CI m/z calcd for $C_4H_9F_3NO$ 144.0636, found 144.0632. Anal. Calcd for $C_4H_8F_3NO$ (corrected for 0.43% w/w of water content as determined by the Karl-Fisher method): C, 33.43; H, 5.67; N, 9.75. Found: C, 33.03; H, 5.94; N, 9.40.

An other major crop was obtained by concentration of the mother liquor under reduced pressure to give the crude desired compound which was used as is in the next step (mixture of diastereomers, 19.4 g).

3-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluorobutan-2-ol (8a). To a solution of the unpurified amino alcohol **7a** (obtained by concentrating the mother liquor) (0.130 mol, 19.4 g) and di-*tert*-butyl dicarbonate (0.130 mol, 29.6 g) in THF (300 mL) and water (150 mL) was added a solution of K_2CO_3 (0.130 mol, 18.7 g) in 60 mL of water. The mixture was vigorously stirred for 48 h, diluted with ethyl acetate (250 mL), and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to give a yellow oil (33.2 g). Chromatography over 200 g of TLC grade silica gel using 20% EtOAc/hexane afforded the desired product **8a** as a crystalline off-white solid (20.8 g, 63%): mp 62−65 °C; ^{1}H NMR (DMSO-d_6, 1:1 mixture of diastereomers) δ 6.84 (d, J = 8.3 Hz, 0.5 H), 6.44 (d, J = 8.9 Hz, 0.5 H), 6.32 (d, J = 6.9 Hz, 0.5 H), 6.23 (d, J = 7.4 Hz, 0.5 H), 3.93−3.81 (m, 1.5 H), 3.73−3.78 (m, 0.5 H), 1.37 (s, 9 H), 1.10 (d, J = 7.4 Hz, 1.5 H), 1.07 (d, J = 6.4 Hz, 1.5 H); ^{13}C NMR (DMSO-d_6, 1:1 mixture of diastereomers) δ 155.5, 155.4, 126.1 (q, J = 285 Hz), 126.0 (q, J = 285 Hz), 78.6, 71.0 (q, J = 28.2 Hz), 46.3, 28.9, 18.1, 16.3; FT-IR (KBr) ν 1680 cm^{-1}; MS CI 244 (MH^+), 261 ($MH^+ + H_2O$). Anal. Calcd for $C_9H_{16}F_3NO_3$: C, 44.44; H, 6.63; N, 5.76. Found: C, 44.51; H, 6.74; N, 5.75.

(12) In the synthesis of HCMV protease inhibitors,[4] this 1:1 mixture was submitted to biological evaluation without separating both diastereomers.

(13) On occasion, a truncated peptide was isolated as a result of the hydrolytic cleavage between the trifluoromethyl ketone-containing residue and the amino acid immediately adjacent to it. This side product was usually observed in cases where the side chains on each side of the hydrolyzed amide bond were unhindered. In most instances, this side product was present in less than 3% yield.

(14) ^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz spectra were recorded on a Bruker AMX 400 spectrometer. FAB mass spectra were recorded on an Autospec, VG spectrometer. Analytical RP-HPLC were performed on a Vydac C18, 5 μm analytical column (15 cm × 4.6 mm) using one of two solvent systems: CH_3CN (0.06% TFA) in H_2O (0.06% TFA) or CH_3CN in 50 mM NaH_2PO_4 at pH 4.4. Amino acid analysis were performed by the PICO-TAG method using a Waters HPLC system. Purification of intermediates was done by flash chromatography on silica gel (Type H, 10−40 μm, Sigma), while peptidyl trifluoromethyl ketones were purified on a semipreparative RP-HPLC column (Whatman column 22 × 500 mm, Partisil 10 ODS, particle size 10 μm, solvent A, H_2O (0.06% TFA); solvent B, 75% CH_3CN/H_2O (0.06% TFA)). Unless otherwise noted, materials were obtained from commercial sources and used without further purification. Protected amino acids were obtained from NovaBiochem, Bachem California Inc., or Advanced Chemtech. Nitrogen protecting groups (α-*N*) were either Boc or Fmoc. Side chain protecting groups on *N*-Fmoc amino acids were *O*-*tert*-butyl (Ser, Thr, Tyr, Asp, and Glu), *N*-trityl (Asn and His), and *N*-Boc (Lys). TBTU was obtained from NovaBiochem and BOP, DIC, and DCC from Aldrich. BHA resin 1% cross-linked with DVB was synthesized in-house (Tam, J. P.; DiMarchi, R. D.; Merrifield, R. B. *Tetrahedron Lett.* **1981**, *22*, 2851). The following abbreviations are used throughout the paper: Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluorenylmethyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DIC, diisopropylcarbodiimide; TBTU, 2-(1 *H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; TFA, trifluoroacetic acid; DIPEA, diisopropylethylamine; NMP, *N*-methylpyrrolidone, BHA·HCl, benzhydrylamine resin hydrochloride salt; TFMK, trifluoromethyl ketone.

(15) Synthesized by a similar protocol as for the isopropyl analogue described in refs 5e,f.

3-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluorobutan-2-one (9a) (Note: the starting material for this reaction (compound **8a**) was synthesized from **4a** as described above but was not purified). To a cold (−60 °C, internal temperature) solution of oxalyl chloride (0.390 mol, 34.1 mL) in anhydrous CH_2Cl_2 (700 mL) was added dropwise over 90 min a solution of DMSO (0.90 mol, 64 mL), dissolved in CH_2Cl_2 (50 mL). After an additional 30 min at −78 °C, a solution of the *unpurified* trifluoromethyl alcohol **8a** (assumed 0.30 mol) in CH_2Cl_2 (200 mL) was added over 2 h. After an extra 30 min at −78 °C, triethylamine was added (0.9 mol, 125 mL), the cooling bath was removed, and the internal temperature was slowly allowed to reach 0 °C over 2 h, at which point, cold water was slowly added. The organic layer was separated and was washed successively with 5% aqueous citric acid, a saturated aqueous solution of $NaHCO_3$, and brine. The solution was dried over Na_2SO_4 and the solvent removed under vacuum to give about 150 mL of a yellow liquid. This liquid was filtered on silica gel (800 g of TLC grade silica gel, 1.5 L of 20% EtOAc/hexane, 1.5 L of 25% EtOAc/hexane, 3 L of 30% EtOAc/hexane) to give the desired trifluoromethyl ketone **9a** as a yellowish pasty solid (36.0 g, 50% overall yield from **4a**). The material was used as such in the next synthetic step; an analytical sample was repurified by silica gel chromatography to give a yellowish solid: mp 79−87 °C; ^{1}H NMR (DMSO-d_6, 80% hydrated form) δ 7.71 (d, J = 6.7 Hz, 0.2 H), 6.85 (s, 0.75 H), 6.77 (s, 0.75 H), 6.35 (d, J = 9.6 Hz, 0.8 H), 4.51 (quintet, J = 6.7 Hz, 0.2 H), 3.86−3.79 (m, 0.8 H), 1.37 (s, 9 H), 1.25 (d, J = 7.0 Hz, 0.6 H), 1.08 (d, J = 6.7 Hz, 2.4 H); ^{13}C NMR (DMSO-d_6, mixture hydrate/non-hydrate) δ 181.5 (d, J = 201 Hz), 154.6, 123.5 (q, J = 291 Hz), 92.8 (q, J = 28.8 Hz), 77.7, 48.6, 27.7, 14.5; FT-IR (KBr) ν 1660, 1530 cm^{-1}; HR-MS CI m/z calcd for $C_9H_{15}F_3NO_3$ 242.1004, found 242.1000. Anal. Calcd for $C_9H_{14}F_3NO_3$ (corrected for 6.61% w/w of water content as determined by the Karl-Fisher method): C, 41.85; H, 6.22; N, 5.42. Found: C, 42.04; H, 6.58; N, 5.47.

***trans*-(4-Semicarbazidomethyl)cyclohexanecarboxylic Acid Benzyl Ester (11).** The Boc-protected semicarbazide **10**[8] (0.143 mol, 58.1 g) was dissolved in 4 N HCl/dioxane (Aldrich) (214 mL). The suspension was stirred with a mechanical stirrer for 1 h, and the mixture was poured into ice water (1 L). Solid K_2CO_3 was added until the pH of the solution reached 11, and the mixture was left standing overnight in the cold. The solid was collected by filtration, washed thoroughly with water, and dried under high vacuum to give the desired compound **11** as a white solid (35.6 g, 81% yield): mp 119−120 °C; ^{1}H NMR (DMSO-d_6) δ 7.35−7.30 (m, 5 H), 6.86 (s, 1 H), 6.32 (broad s, 1 H), 5.07 (s, 2 H), 4.09 (s, 2 H), 2.87 (t, J = 6.4 Hz, 2 H), 2.28 (tt, J = 12.2 and 3.4 Hz, 1 H), 1.92 (dd, J = 13.2 and 2.9 Hz, 2 H), 1.71 (dd, J = 13.3 and 2.9 Hz, 2 H), 1.37−1.24 (m, 3 H), 0.90 (dq, J = 13.0 and 3.2 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 174.3, 159.7, 135.8, 127.9, 127.3, 127.1, 64.6, 44.3, 42.0, 37.0, 28.6, 27.7; IR (KBr) ν 1720, 1645 cm^{-1}; FAB-MS m/z 306. Anal. Calcd for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76. Found: C, 63.10; H, 7.81; N, 13.77.

Semicarbazone 12a. A suspension of compound **9a** (114 mmol, 27.4 g) and of compound **11** (114 mmol, 34.7 g) in toluene (300 mL) was refluxed for 3 h in the presence of 5 mol % of *p*-toluenesulfonic acid monohydrate (5.7 mmol, 1.1 g). The mixture was then cooled, diluted with EtOAc (300 mL), and washed successively with 1 M aqueous HCl, a saturated aqueous solution of $NaHCO_3$, a 0.6% aqueous solution of bleach, a 5% aqueous solution of sodium thiosulfate, and brine. The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to half its initial volume, at which point compound **12a** precipitated from the mixture. This solid was collected by filtration, washed with several portions of 6:4 EtOAc/hexane and dried under vacuum to give **12a** as a white solid (22.1 g, 37% yield): mp 162.5−163 °C; ^{1}H NMR (DMSO-d_6) δ 10.24 (s, 1 H), 7.50 (broad s, 1 H), 7.39−7.29 (m, 5 H), 6.80 (t, J = 6.8 Hz, 1 H), 5.07 (s, 2 H), 4.77−4.70 (m, 1 H), 2.99 (t, J = 6.4 Hz, 2 H), 2.30 (tt, J = 12.0 and 3.3 Hz, 1 H), 1.92 (broad d, J = 10.8 Hz, 2 H), 1.71 (broad d, J = 11.2 Hz, 2 H), 1.43−1.27 (m, 12 H), 1.19 (d, J = 7.6 Hz, 3 H), 0.93 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 175.6, 155.5, 137.2, 136.7 (quartet, J = 29.5 Hz), 129.3, 128.7, 128.5, 121.9 (quartet, J = 277 Hz), 79.3, 66.0, 45.8, 43.5, 43.3, 38.1, 30.0, 29.0, 28.9; FT-IR (KBr) ν 1726, 1690 cm^{-1}; CI-MS m/z 529. Anal. Calcd for $C_{25}H_{35}F_3N_4O_5$: C, 56.81; H, 6.67; N, 10.60. Found: C, 56.63; H, 6.69; N, 10.62.

A second crop of **12a** was obtained after concentration of the mother liquors (8.91 g, 15% yield). The ^{1}H NMR was also consistent with the assigned structure but contained ∼12 mol % of the *Z* isomer. Total yield: 31 g, 52%.

Semicarbazone 13a. A suspension of compound **12a** (21.9 g, 41.4 mmol) in EtOAc (300 mL) and 95% EtOH (300 mL) was hydrogenated overnight at 45 psi on a Parr apparatus over 5% w/w Pd on carbon (2.2 g). At this point, TLC analysis indicated complete consumption of the starting material. Since the acid was not soluble in this solvent system, the mixture was thoroughly degassed with nitrogen and diluted with CH_2Cl_2 (200 mL) and MeOH (500 mL). The medium was gently refluxed for 30 min on a water bath; the catalyst was removed by filtration on Celite and washed with several 20 mL portions of CH_2Cl_2 followed by 20 mL portions of MeOH. The solvent was removed under reduced pressure, and the resulting white solid **13a** was triturated with 250 mL of Et_2O and filtered (18.0 g, 99% yield): mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 11.96 (s, 1 H), 10.20 (s, 1 H), 7.47 (broad, 1 H), 6.77 (t, J = 5.9 Hz, 1 H), 4.74 (quintet, J = 6.9 Hz, 1 H), 3.31 (broad s, 1 H), 2.99 (t, J = 6.4 Hz, 2 H), 2.11 (tt, J = 12.0 and 0.8 Hz, 1 H), 1.89 (broad d, J = 10.3 Hz, 2 H), 1.70 (broad d, J = 10.8 Hz, 2 H), 1.36 (s, 9 H), 1.30−1.19 (m, 5 H), 0.91 (double quartet, J = 12.5 and 2.6 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 175.7, 153.9, 153.6, 134.9 (quartet, J = 29 Hz), 120.1 (quartet, J = 276 Hz), 77.5, 44.0, 41.7, 41.5, 36.3, 28.3, 27.2, 27.0, 15.0; FT-IR (KBr) ν 1700, 1540 cm^{-1}; CI-MS m/z 439. Anal. Calcd for $C_{18}H_{29}F_3N_4O_5$: C, 49.31; H, 6.67; N, 12.78. Found: C, 48.93; H, 6.81; N, 12.52.

Resin 3a. To a suspension of a BHA·HCl resin (loading: 0.71 mmol/g, 18.1 mmol, 25.4 g) in DMSO (75 mL) and NMP (400 mL) were added **13a** (23.5 mmol, 10.3 g), DIPEA (72.4 mmol, 12.6 mL), anhydrous HOBt (23.5 mmol, 3.17 g), and TBTU (23.5 mmol, 7.55 g). The slurry was stirred with a mechanical stirrer for 15 h at which point the Kaiser test was negative. The resin was filtered and was washed successively with 300 mL portions of DMF (1×), CH_2Cl_2 (2×), MeOH (1×), CH_2Cl_2 (2×), MeOH (2×), CH_2Cl_2 (2×), and MeOH (2×). The resulting resin **3a** was dried by suction under a nitrogen atmosphere and then under high vacuum overnight. Titration by the picric acid method[16] gave a loading of 0.42 mmol/g.

3-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluoropentan-2-ol (8b). This compound was prepared in 64% yield from nitropropane using the procedure described above for compound **8a**. **8b**: mp 78−79 °C; ^{1}H NMR (DMSO-d_6, 3:2 mixture of diastereomers) δ 6.72 (d, J = 9.2 Hz, 0.60 H), 6.28 (d, J = 7.3 Hz, 0.60 H), 6.25 (d, J = 9.9 Hz, 0.4 H), 6.18 (d, J = 7.6 Hz, 0.4 H), 3.95−3.91 (m, 0.4 H), 3.90−3.75 (m, 0.6 H), 3.69−3.62 (m, 0.4 H), 3.55−3.48 (m, 0.6 H), 1.69−1.64 (m, 0.6 H), 1.56−1.37 (m, 1.4 H), 1.37 (s, 9 H), 0.86−0.80 (quartet, J = 7.8, 3 H); ^{13}C NMR (DMSO-d_6) 155.5, 125.8 (quartet, J = 284 Hz), 125.5 (quartet, J = 284 Hz), 77.9, 77.8, 71.1−69.5 (m), 51.4, 51.2, 28.3, 28.1, 24.5, 22.7, 10.6, 10.0; IR (KBr) ν 1676 cm^{-1}; HR-MS FAB m/z calcd for $C_{10}H_{19}F_3NO_3$ 258.1317, found 258.1310. Anal. Calcd for $C_{10}H_{18}F_3NO_3$: C, 46.69; H, 7.05; N, 5.44. Found: C, 46.99; H, 6.96; N, 5.45.

3-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluoropentan-2-one (9b) (Note: the starting material for this reaction (compound **8b**) was synthesized from **4b** as described above but was not purified). The title compound was prepared in 45% yield from **4b** using the procedure described above for compound **9a**. **9b**: mp 50−53 °C; ^{1}H NMR (DMSO-d_6, 75% hydrated form) δ 7.66 (d, J = 6.7 Hz, 0.75 H), 6.79 (s, 0.25 H), 6.66 (s, 0.25 H), 6.22 (d, J = 10.2 Hz, 0.25 H), 4.37−4.32 (m, 0.75 H), 3.62−3.57 (m, 0.25 H), 1.81−1.71 (m, 1 H), 1.66−1.53 (m, 0.75 H), 1.38 and 1.32 (s, 9.25 H), 0.90 (t, J = 7.5 Hz, 2.25 H), 0.82 (t, J = 7.5 Hz, 0.75 H); ^{13}C NMR (DMSO-d_6), 190.8 (quartet, J = 32 Hz), 155.9, 155.8, 124.2 (quartet, J = 291 Hz), 115.6 (quartet, J = 295 Hz), 93.4 (quartet, J = 29 Hz), 79.1, 78.0, 57.0, 55.1, 28.3, 28.1, 27.8, 22.1, 20.9, 10.5, 10.1; IR (KBr) ν 1660 cm^{-1}; HR-MS CI m/z calcd for $C_{10}H_{17}F_3NO_3$ 256.1160, found 256.1168. Anal. Calcd for $C_{10}H_{16}F_3NO_3$ (corrected for 3.64% w/w of water content as determined by the Karl-Fisher method): C, 45.34; H, 6.51; N, 5.29. Found: C, 45.05; H, 6.94; N, 5.33.

(16) Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248.

Semicarbazone 12b. This compound was prepared in 58% yield from **9b** using the procedure described above for compound **12a**. **12b**: mp 165−166 °C; ^{1}H NMR (DMSO-d_6) δ 10.29 (s, 1 H), 7.41−7.29 (m, 6 H), 6.76 (t, J = 6.0 Hz, 1 H), 5.08 (s, 2 H), 4.62−4.60 (m, 1 H), 2.99 (broad t, J = 5.9 Hz, 2 H), 2.30 (tt, J = 12.1 and 3.4 Hz, 1 H), 1.93 (broad d, J = 13.0 Hz, 2 H), 1.71 (broad d, J = 11.8 Hz, 2 H), 1.67−1.58 (m, 1 H), 1.55−1.48 (m, 1 H), 1.44−1.42 (m, 1 H), 1.37 (s, 9 H), 1.35−1.26 (m, 2 H), 0.99−0.87 (m, 2 H), 0.89 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (DMSO-d_6) 175.0, 155.4, 154.9, 136.6, 135.3 (quartet, J = 29 Hz), 128.6, 128.1, 127.8, 121.3, (quartet, J = 277 Hz), 78.6, 65.3, 48.5, 45.2, 42.6, 37.4, 29.3, 28.3, 28.2, 23.2, 10.2; IR (KBr) ν 1730, 1666 cm^{-1}; HR-MS FAB m/z calcd for $C_{26}H_{38}F_3N_4O_5$ 543.2794, found 543.2777. Anal. Calcd for $C_{26}H_{37}F_3N_4O_5$: C, 57.55; H, 6.87; N, 10.33. Found: C, 57.84; H, 7.03; N, 10.40.

Semicarbazone 13b. This compound was prepared in 95% yield from **12b** using the procedure described above for compound **13a** ($Pd(OH)_2$ was used as catalyst). **13b**: mp 224−225 °C; ^{1}H NMR (DMSO-d_6) (4:1) mixture of Z and E, δ 11.96 (s, 1 H), 10.29 (s, 1 H), 7.40 (broad d, J = 5.4 Hz, 0.80 H), 7.01 (broad s, 0.20 H), 6.75 (t, J = 6.0 Hz, 1 H), 4.65−4.55 (m, 1 H), 3.05−2.95 (m, 2 H), 2.16−2.06 (m, 1 H), 1.89 (broad d, J = 10.8 Hz, 2 H), 1.71 (broad d, J = 11.5 Hz, 2 H), 1.66−1.58 (m, 1 H), 1.56−1.46 (m, 1 H), 1.37 (s, 10 H), 1.25 (quartet, J = 11.5 Hz, 2 H), 0.97−0.85 (m, 2 H), 0.89 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (DMSO-d_6), 176.9, 155.5, 154.8, 135.3 (quartet, J = 29 Hz), 121.4 (quartet, J = 278 Hz), 78.6, 48.5, 45.2, 42.7, 37.5, 35.9, 29.5, 28.4, 28.3, 23.2, 10.2; IR (KBr) ν 1682 cm^{-1}; HR-MS MS m/z calcd for $C_{19}H_{32}F_3N_4O_5$ 453.2325, found 453.2333. Anal. Calcd for $C_{19}H_{31}F_3N_4O_5$: C, 50.44; H, 6.91; N, 12.38. Found: C, 50.50; H, 7.02; N, 12.67.

Resin 3b. This resin was prepared from BHA·HCl and compound **13b** by a procedure similar to that described for the preparation of resin **3a**. Loading: 0.36 mmol/g.

4-Methyl-3-[(*tert*-butoxycarbonyl)amino]-1,1,1-trifluoropentan-2-ol (8c).[5e] This compound was prepared in 26% yield from 2-methyl-1-nitropropane[17] using the procedure described above for compound **8a**. **8c**: mp 100−104 °C; ^{1}H NMR (DMSO-d_6, 4:5 mixture of diastereomers) δ 6.66 (d, J = 9.6 Hz, 0.45 H), 6.33 (d, J = 7.3 Hz, 0.55 H), 6.22−6.19 (m, 1 H), 4.09−4.02 (m, 0.55 H), 3.83−3.63 (m, 0.9 H), 3.50−3.40 (m, 0.55 H), 2.32−2.06 (m, 0.45 H), 1.81−1.72 (m, 0.55 H), 1.37 (s, 9 H), 0.88 (d, J = 6.6 Hz, 1.5 Hz), 0.85 (d, J = 6.7 Hz, 1.5 H), 0.78 (d, J = 7.0 Hz, 1.5 H), 0.73 (d, J = 6.7 Hz, 1.5 H); ^{13}C NMR (DMSO-d_6) δ 155.4, 155.1, 126.7 (q, J = 285 Hz), 126.0 (q, J = 282 Hz), 78.4, 78.2, 69.2−67.9 (m), 54.7, 53.5, 30.6, 28.8, 28.6, 20.2, 20.1, 15.7; IR (KBr) ν 1670 cm^{-1}; HR-MS MS m/z calcd for $C_{11}H_{21}F_3NO_3$ 272.1473, found 272.1485. Anal. Calcd for $C_{11}H_{20}F_3NO_3$: C, 48.70; H, 7.43; N, 5.16. Found: C, 48.82; H, 7.50; N, 5.22.

4-Methyl-3-[(*tert*-butoxycarbonyl)amino]-1,1,1-trifluoropentan-2-one (9c). This compound was prepared in 84% yield from **8c** using the procedure described above for compound **9a**. **9c**: mp 51−54 °C; ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 7.2 Hz, 0.9 H), 7.29 (broad s, 0.1 H), 4.30 (t, J = 6.9 Hz, 1 H), 2.19−2.11 (m, 1 H), 1.39 (s, 8.1 H), 1.34 (s, 0.9 H), 0.92−0.88 (m, 6 H); ^{13}C NMR (DMSO-d_6) δ 193.0 (quartet, J = 32 Hz), 158.2, 117.6 (quartet, J = 294 Hz), 81.3, 64.4, 63.1, 30.3, 23.3, 30.0, 29.0, 20.0, 19.3; IR (KBr) ν 1753, 1720 cm^{-1}; HR-MS CI m/z calcd for $C_{11}H_{19}F_3NO_3$ 270.1317, found 270.1326. Anal. Calcd for $C_{11}H_{18}F_3NO_3$: C, 49.07; H, 6.74; N, 5.20. Found: C, 48.75; H, 6.76; N, 5.14.

Semicarbazone 12c. This compound was prepared in 72% yield from **9c** using the procedure described above for compound **12a**. **12c**: mp 65−70 °C; ^{1}H NMR (DMSO-d_6) δ 10.34 (broad s, 1 H), 7.40−7.30 (m, 6 H), 6.73 (t, J = 6.0 Hz, 1 H), 5.08 (s, 2 H), 4.45 (broad s, 1 H), 2.99 (broad s, 2 H), 2.35−2.25 (m, 1 H), 1.93 (broad d, J = 12.7 Hz, 3 H), 1.71 (broad d, J = 12.1 Hz, 2 H), 1.45−1.25 (m, 12 H), 1.05−0.90 (m, 5 H), 0.79 (d, J = 6.7 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 176.6, 157.0, 156.5, 156.1, 138.2, 136.2 (quartet, J = 29.6 Hz), 130.2, 129.7, 129.5, 125.7 (quartet, J = 276 Hz), 80.1, 67.0, 55.4, 54.6, 46.9, 44.3, 39.0, 31.0, 30.7, 30.0, 29.9, 29.6, 21.3, 19.7; IR (KBr) ν 1721, 1680 cm^{-1}; HR-MS FAB m/z calcd for $C_{27}H_{40}F_3N_4O_5$ 557.2951, found 557.2931. Anal. Calcd for $C_{27}H_{39}F_3N_4O_5$: C, 58.26; N, 10.07; H, 7.06. Found: C, 58.08; N, 9.71; H, 7.04.

Semicarbazone 13c. This compound was prepared in 87% yield from **12c** using the procedure described above for compound **13a**. **13c**: mp 115−125 °C; ^{1}H NMR (DMSO-d_6) δ 12.00 (s, 1 H), 10.39 (broad s, 0.3 H), 10.34 (s, 0.7 H), 7.40 (d, J = 6.4 Hz, 0.7 H), 7.01 (broad, 0.3 H), 6.72 (t, J = 6.2 Hz, 1 H), 4.45 (broad s,1 H), 2.99 (broad s, 2 H), 2.11 (t, J = 12.0 Hz, 1 H), 1.91 (broad s, 1 H), 1.89 (broad d, J = 10.8 Hz, 2 H), 1.70 (broad d, J = 11.3 Hz, 2 H), 1.37 (s, 9 H), 1.32−1.20 (m, 3 H), 1.05−0.85 (m, 2 H), 0.79 (d, J = 6.4 Hz, 6 H); ^{13}C NMR: (DMSO-d_6) δ 176.2, 154.7, 154.1, 133.8 (quartet, J = 30 Hz), 120.7 (quartet, J = 276 Hz), 77.8, 59.2, 53.0, 52.2, 44.6, 36.8, 28.8, 28.3, 27.7, 27.6, 27.3, 18.9, 17.4, 13.5; IR (KBr) ν 1697 cm^{-1}; HR-MS FAB m/z calcd for $C_{20}H_{34}F_3N_4O_5$ 467.2481, found 467.2492. Anal. Calcd for $C_{20}H_{33}F_3N_4O_5$: C, 51.49; N, 12.01; H, 7.13. Found: C, 51.74; N, 12.01; H, 7.52.

Resin 3c. This resin was prepared from BHA·HCl and compound **13c** by a procedure similar to that reported for the preparation of resin **3a**. Loading: 0.44 mmol/g.

General Procedures for the Parallel Peptide Synthesis. The peptides were assembled on an ACT396 peptide synthesizer (protocols 1, 2, and 4) or on an ACT90 peptide synthesizer (protocol 3). Each reaction vessel was charged with the appropriate resin **3a**−**c** (0.25 mmol) which was washed thoroughly with several portions of CH_2Cl_2 and treated with a 45% solution of TFA in CH_2Cl_2 (2×, 5 min then 20 min). The resins were then washed successively with CH_2Cl_2 (2×), 5% DIPEA in CH_2Cl_2 (2×, 1 min and then 5 min), CH_2Cl_2 (3×), MeOH (2×), and CH_2Cl_2 (3×). The subsequent peptide synthesis was done following different protocols depending on the type of amino acid protection, coupling agent, and equipment used.

Protocol 1: Boc/DIC/HOBt. The resin was suspended in CH_2Cl_2 (0.35 mL) and was treated with a 0.5 M solution of the Boc-protected amino acid dissolved in 0.5 M HOBt in DMF (2.4 mL, 1.2 mmol of each), followed by a 0.5 M DIC solution in CH_2Cl_2 (2.4 mL, 1.2 mmol). The reaction was allowed to proceed for 3.5 h and was repeated once (double coupling). After the coupling, the resin was washed successively with 5 mL portions of CH_2Cl_2 (3×), MeOH (2×), and CH_2Cl_2 (3×). Before addition of the next amino acid, the Boc protecting group was removed with a 45% solution of TFA in CH_2Cl_2 (25 min), and the resin was neutralized with 5% DIPEA in CH_2Cl_2 and was washed as described above.

Protocol 2: Fmoc/TBTU/HOBt. The resin was suspended in NMP (0.35 mL) and was treated with a 0.5 M solution of the Fmoc-protected amino acid dissolved in 0.5 M HOBt in DMF (1.8 mL, 0.9 mmol of each), followed by a 0.5 M TBTU solution in DMF (1.8 mL, 0.9 mmol) and by a 1.0 M solution of DIPEA in NMP (1.8 mL, 1.8 mmol). The reaction was allowed to proceed for 1.25 h and was repeated once (double coupling). After the coupling, the resin was washed successively with 3.5 mL portions of DMF (3×), MeOH (2×), and DMF (3×). Before addition of the next amino acid, the Fmoc protecting group was removed with a 25% solution of piperidine in DMF (3.5 mL for 25 min) and was washed as above.

Protocol 3: Boc/TBTU/HOBt. The resin was suspended in DMF (15 mL) and was treated with the Boc-protected amino acid (0.75 mmol), HOBt hydrate (0.75 mmol, 115 mg), TBTU (0.75 mol, 241 mg), and DIPEA (1.5 mmol, 0.26 mL). The coupling was allowed to proceed for 1 h and was repeated once in cases in which the Kaiser test indicated incomplete reaction. After the coupling, the resin was washed successively with CH_2Cl_2 (3×), MeOH (2×), and CH_2Cl_2 (3×). Before addition of the next amino acid, the Boc protecting group was removed with a 45% solution of TFA in CH_2Cl_2 (15 mL for 25 min), and the resin was neutralized with 5% DIPEA in CH_2Cl_2 and was washed as described above.

Protocol 4: Boc/BOP. The resin was suspended in CH_2Cl_2 (0.35 mL) and was treated with a 0.5 M solution of the Boc-protected amino acid in CH_2Cl_2 (1.5 mL, 0.75 mmol), a 0.5 M BOP solution in CH_2Cl_2 (1.5 mL, 0.75 mmol), and a 1.0 M solution of DIPEA in CH_2Cl_2 (1.5 mL, 1.5 mmol). Each coupling was allowed to proceed for 4 h and was repeated once (double coupling). After the coupling, the resin was washed successively with 5 mL portions of CH_2Cl_2 (2×), MeOH (2×), and CH_2Cl_2 (2×). Before addition of the next amino acid, the Boc protecting groups

(17) Kornblum, N.; Taub, B.; Ungnade, H. E. *J. Am. Chem. Soc.* **1954**, *76*, 3209.

was removed with a 45% solution of TFA in CH_2Cl_2 (25 min), and the resin was neutralized with 5% DIPEA in CH_2Cl_2 and was washed as above.

General Procedure for the Cleavage of the Peptidyl Trifluoromethyl Ketones from the Solid Support. In cases in which acid-sensitive side chain protecting groups were present, these were removed before releasing the peptide from the resin by treatment with a solution containing 75% TFA and 5% anisole in CH_2Cl_2 for 3 h.[18] The resin was then washed thoroughly with CH_2Cl_2 (2×), MeOH (2×), CH_2Cl_2 (2×), and finally with MeOH (3×). The dried resin (~800 mg) was suspended in THF (9 mL), H_2O (0.50 mL), AcOH (0.25 mL), and 1 M aqueous HCl (0.12 mL) and was heated in a sealed tube at 65 °C for 4 h. The solution was cooled, filtered, and treated as above at least once more. The total number of treatments were determined on a case by case basis by estimating the amount of compound released after each repeat by HPLC analysis of the mother liquor. In cases in which the sequence contained basic residues such as lysine or histidine, an extra 0.06 mL of 1 M HCl was used and the procedure was repeated a third time (see Table 1 for total number of treatments). All the mother liquors were combined, the THF was concentrated in vacuo, and the residue was purified by reversed-phase HPLC. The desired peptidyl trifluoromethyl ketones **14**−**28** were isolated in the yields reported in Table 1.

(18) Compound **19** was treated in a different way (see Supporting Information).

Acknowledgment. The authors are grateful to N. Aubry for performing the NOE experiments on compounds **12a** and **12c** and to S. Valois, S. Bordeleau, and C. Boucher for their analytical support. Drs. P. Lavallée and W. W. Ogilvie are also acknowledged for their assistance during the preparation of this manuscript.

Supporting Information Available: ^{1}H NMR, HR-MS, amino acid analysis, and HPLC homogeneity of compounds **14**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9815204