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Fluorinated linkers for monitoring solid-phase synthesis using gel-phase ^{19}F NMR spectroscopy

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Abstract: Three fluorinated linkers which are analogues of linkers commonly used in solid-phase peptide synthesis have been prepared. Using ^{19}F NMR spectroscopy, the fluorine atom of the linker allowed monitoring of several transformations in the solid-phase synthesis of a peptoid having a coumarin moiety. Especially, attachment of the linker to the solid phase, coupling of the first building block to the linker and cleavage of the product were efficiently monitored and optimised.

Organic synthesis performed on solid phase constitutes an efficient method for preparation of large combinatorial libraries containing structurally distinct molecules [1-3]. It is presently the focus of substantial interest due to its impact on both lead structure identification and optimisation in pharmaceutical research. Adaption of organic reactions, as well as analytical techniques, so as to become compatible with various solid supports are important areas of solid-phase organic synthesis that require continuous development.

A key aspect of any solid-phase synthesis is the choice of linker [4]. It should be orthogonal to the required reaction conditions and allow quantitative cleavage of the product under mild conditions. In addition it would be an advantage if the linker also contained features that served analytical purposes. In view of our previous work with gel phase ^{19}F NMR spectroscopy [5], we realized the potential in using fluorinated linkers to monitor reactions on solid phase and now describe synthesis of three such linkers. One of the linkers (**12**) was employed for preparation of a peptoid having a coumarin moiety (**18**), a member of a library of compounds designed as inhibitors of the chaperone PapD which is required for pilus assembly in uropathogenic *E. coli* [6].

The three linkers are fluorinated analogues of linkers commonly used in solid-phase peptide synthesis (Schemes 1 and 2) [7]. *p*-Hydroxymethylbenzoic acid type linkers (cf. **2**) are essentially acid-stable, but are cleaved under basic or nucleophilic conditions. Linkers based on 3-[4-(hydroxymethylphenyl)]alkanoic acids (cf. **7**) require strongly acidic conditions for cleavage (*e.g.* liquid hydrogen fluoride), whereas 4-(hydroxymethyl)phenoxyacetic acid linkers (cf. **12**) are cleaved by milder acids such as trifluoroacetic acid. Linkers **2** and **7** were prepared from dimethyl-2-fluoroterephtalate (**1**, Scheme 1). Non-selective, basic hydrolysis of one of the ester moieties of **1**, followed by reduction of the remaining ester with $LiBH_4$ and chromatographic separation of the two regioisomers gave the fluorinated 4-

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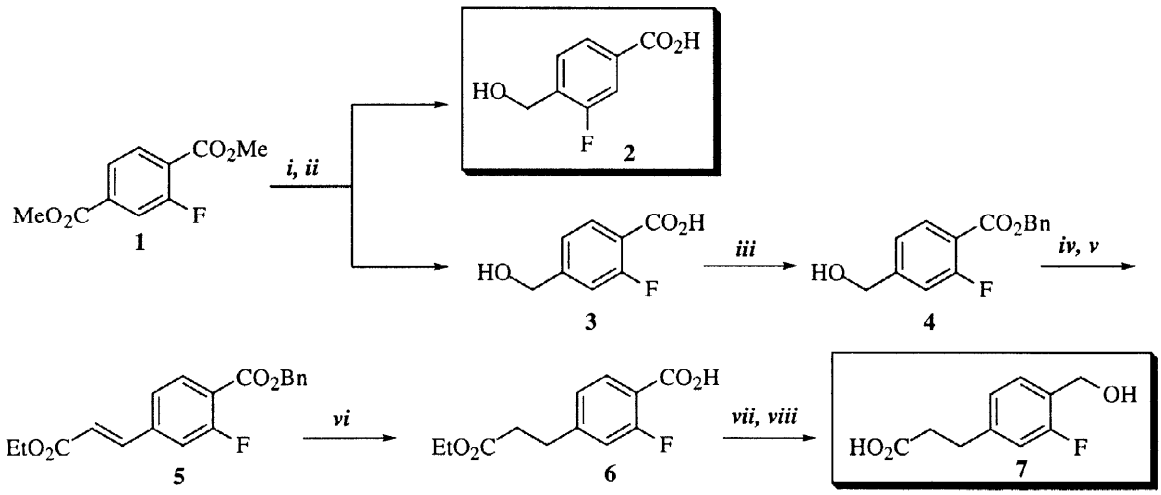
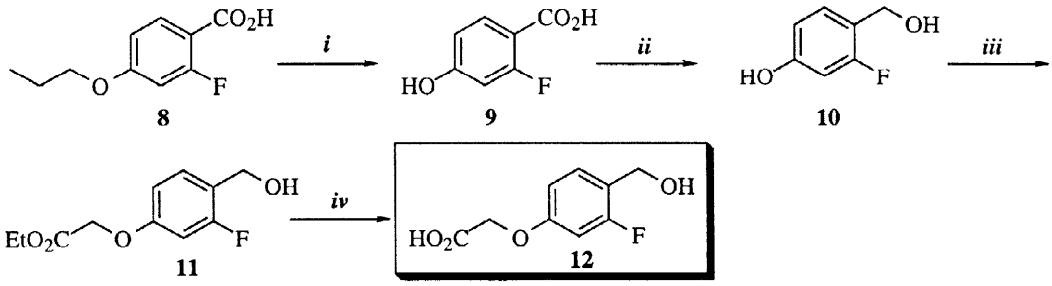
(hydroxymethyl)benzoic acids **2** [8] and **3**. Protection of **3** as a benzyl ester, followed by Swern oxidation and condensation of the resulting aldehyde with triethyl phosphonoacetate afforded **5** which was reduced to **6**. Reduction of the carboxyl group of **6** using BH_3-DMS and $(MeO)_3B$ followed by hydrolysis of the ethyl ester then furnished linker **7** [9]. Linker **12** was prepared by BBr_3-induced dealkylation of 2-fluoro-4-propoxybenzoic acid (**8**), followed by reduction with BH_3-DMS and $(MeO)_3B$ to give hydroxymethylphenol **10** (Scheme 2). *O*-Alkylation of **10** with bromoethyl acetate and DBU as a base gave ester **11**, and subsequent treatment with LiOH afforded **12** [10] in good overall yield.



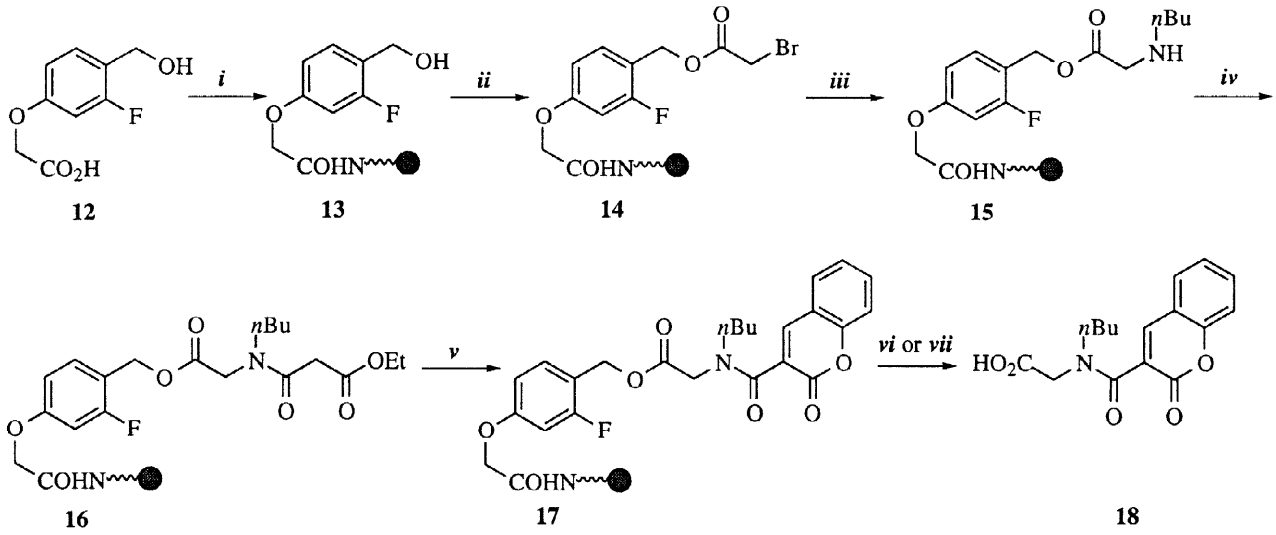
Scheme 1: *Reaction conditions*: (*i*) 1M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C→r.t.; (*ii*) $LiBH_4$, THF, **2** 41% and **3** 31% from **1**; (*iii*) 20% aq. Cs_2CO_3, MeOH:H_2O (10:1), then BnBr, DMF, 79%; (*iv*) TPAP (5 mol%), *N*-methylmorpholine *N*-oxide, 4Å molecular sieves, CH_2Cl_2, 74%; (*v*) NaH, $(EtO)_2P(O)CH_2CO_2C_2H_5$, THF, 0 °C→r.t., 76%; (*vi*) 10% Pd/C, H_2, EtOH:EtOAc (3:1), 4 atm., 88%; (*vii*) BH_3-DMS, $(MeO)_3B$, THF, 89%; (*viii*) 1M LiOH, THF:MeOH:H_2O (3:1:1), 0 °C→r.t., 93%.



Scheme 2: *Reaction conditions*: (*i*) BBr_3, CH_2Cl_2, -78 °C→r.t., 89%; (*ii*) BH_3-DMS, $(MeO)_3B$, THF, 90%; (*iii*) $BrCH_2CO_2C_2H_5$, DBU, CH_3CN, reflux, 74%; (*iv*) 1M LiOH, THF:MeOH:H_2O (3:1:1) 87%.

3-Fluoro-4-(hydroxymethyl)phenoxyacetic acid (**12**) was then used as linker in solid phase synthesis of peptoid **18** (Scheme 3). In brief, activation of **12** as a pentafluorophenyl ester allowed coupling to TentaGel S NH_2 resin and subsequent acylation of the resulting benzylic alcohol **13** with bromoacetic acid afforded **14**. Nucleophilic substitution of the

bromoacetate with *n*-butylamine followed by amidation of **15** with ethyl malonyl chloride gave **16**. Knoevenagel condensation of **16** with salicylaldehyde and cleavage of the product from the solid phase under basic conditions, furnished **18** in 55% overall yield based on the capacity of the resin.



Scheme 3: *Reaction conditions:* (*i*) Pentafluorophenol, DIC, TentaGel S NH_2, EtOAc; (*ii*) $BrCH_2CO_2H$, DIC, HOBt, DMAP, THF; (*iii*) *n*-Butylamine, CH_3CN, 0 °C; (*iv*) $ClCOCH_2CO_2C_2H_5$, DIPEA, CH_2Cl_2, 0 °C; (*v*) Salicylaldehyde, piperidine, CH_3CN, reflux; (*vi*) TFA:H_2O 2:1, 27 % overall yield based on resin capacity, or (*vii*) 1M LiOH, THF:MeOH:H_2O (3:1:1), 55% overall yield based on resin capacity.

Based on the fluorine atom found in the linker several of the steps leading to **18** could be efficiently monitored and then optimised by use of ^{19}F NMR spectroscopy [11]. It should be pointed out that high quality spectra were obtained within minutes for samples of resin in an ordinary NMR tube using a standard NMR spectrometer. When linker **12** was coupled to the TentaGel resin, using 1-hydroxy-7-azabenzotriazole (HOAt) and diisopropylcarbodiimide, a tendency for twofold coupling of the linker was revealed, *i.e.* after formation of **13** the linker coupled to the hydroxyl group of this derivative [12]. This problem could be circumvented by using milder reaction conditions, *i.e.* coupling of **12** as the pentafluorophenyl ester. In acylation of **13** with bromoacetic acid it was found that the acylation had to be repeated in order to obtain complete conversion into **14** (Figure 1). Moreover, when **16** was transformed into **17** it was detected that 20% of the product was cleaved from the resin during the condensation (Figure 2a). Finally, ^{19}F NMR spectroscopy proved to be particularly efficient when monitoring the cleavage of the product from the solid support to give **18**. Cleavage under acidic conditions with TFA:H_2O (2:1), provided **18** in only 27% overall yield (based on the resin capacity), while a large part of **17** remained unaffected. In contrast, treatment with 1M LiOH accomplished complete cleavage and furnished **18** in 55% overall yield (Figure 2b).

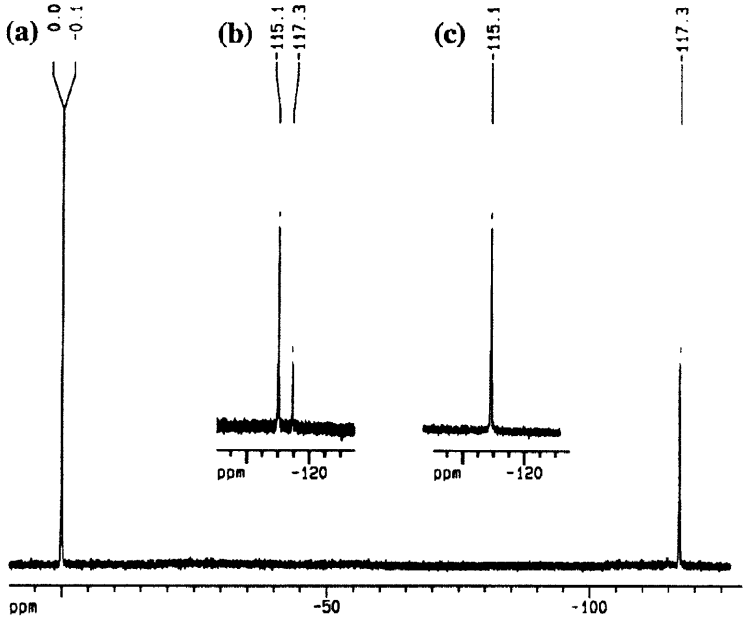


Figure 1. Monitoring of the transformation of **13** into **14** by ^{19}F NMR spectroscopy; (a) **13**, (b) acylation of **13** with 3 equivalents of bromoacetic acid gave only partial conversion into **14**, and (c) full conversion was obtained after a second acylation.

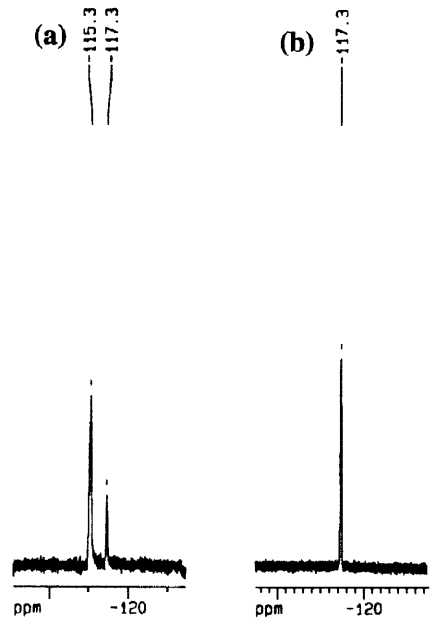


Figure 2. ^{19}F NMR revealed (a) that 20% of the material was lost from the solid phase during transformation of **16** into **17**, and (b) that treatment with 1M LiOH for 2.5 h resulted in complete cleavage of **18** from the solid support.

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REFERENCES AND NOTES

[1] Balkenhohl F, von dem Bussche-Hünnefeld C, Lansky A, Zechel C. Angew. Chem. Int. Ed. Engl. 1996;35:2288-2337.

[2] Früchtel JS, Jung G. Angew. Chem. Int. Ed. Engl. 1996;35:17-42.

[3] Thompson LA, Ellman JA. Chem. Rev. 1996;96:555-600.

[4] Fields GB, Tian Z, Barany G. Principles and Practice of Solid-Phase Peptide Synthesis. In: Grant GA, editor. Synthetic Peptides: A User's Guide. New York: Freeman WH and Company, 1992:77-183.

[5] Svensson A, Fex T, Kihlberg J. Tetrahedron Lett. 1996;37:7649-7652.

[6] Flemmer Karlsson K, Walse B, Drakenberg T, Roy S, Bergquist K-E, Pinkner JS, Hultgren SJ, Kihlberg J. Bioorg. Med. Chem., submitted .

[7] Sheppard RC, Williams BJ. Int. J. Peptide Protein Res. 1982;20:451-454.

[8] 3-Fluoro-4-(hydroxymethyl)benzoic acid (**2**). ^1H NMR (CD_3OD, 400 MHz) δ 7.83 (dd, 1H, *J*=7.9, 1.4 Hz, Ar), 7.64 (dd, 1H, *J*=10.6, 1.4 Hz, Ar), 7.58 (t, 1H, *J*=7.6 Hz, Ar), 4.73 (s, 2H, ArCH_2OH); ^{13}C NMR (100 MHz) δ 168.6, 162.6, 160.1, 135.2, 130.1, 126.7, 117.2, 58.7; HRMS calcd. for $C_8H_7O_3F$: 170.0379; found: 170.0374.

[9] 3-[3-Fluoro-4-(hydroxymethylphenyl)]propionic acid (**7**). ^1H NMR (CD_3OD, 400 MHz) δ 7.35 (t, 1H, *J*=7.8 Hz, Ar), 7.04 (dd, 1H, *J*=7.8, 1.5 Hz, Ar), 6.96 (dd, 1H, *J*=11.2, 1.5 Hz), 4.62 (s, 2H, ArCH_2OH), 2.91 (t, 2H, *J*=7.5 Hz, ArCH_2CH_2), 2.60 (t, 2H, *J*=7.5 Hz, ArCH_2CH_2); ^{13}C NMR (100 MHz) δ 175.4, 162.0, 159.8, 143.2, 129.6, 124.2, 115.0, 57.7, 35.3, 30.4; HRMS calcd. for $C_{10}H_{11}O_3F$: 198.0692; found; 198.0692.

[10] 3-Fluoro-4-(hydroxymethyl)phenoxyacetic acid (**12**). ^1H NMR (CD_3OD, 400 MHz) δ 7.34 (t, 1H, *J*=8.6 Hz, Ar), 6.76 (ddd, 1H, *J*=8.3, 2.5, 0.8 Hz, Ar), 6.70 (dd, 1H, *J*=11.8, 2.5 Hz, Ar), 4.66 (s, 2H, ArOCH_2), 4.58 (s 2H, ArCH_2OH); ^{13}C NMR (100 MHz) δ 172.4, 163.8, 161.4, 160.4, 131.6, 122.5, 111.4, 103.5, 66.2, 58.6; HRMS calcd. for $C_9H_9O_4F$: 200.0484; found 200.0486.

[11] Gel phase ^{19}F NMR spectra were recorded with a Bruker ARX-400 spectrometer operating at 375.5 MHz for solutions in $CDCl_3$ with Cl_3CF (δ_F 0.0 ppm) as internal standard.

[12] A by-product was obtained from the double-coupled linker during cleavage of **18** from the solid phase. Separation of this by-product from **18** was difficult, underscoring the need for attaching only one equivalent of the linker to the solid phase.