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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 6177–6180

Synthesis of a fluoroalkene peptidomimetic precursor of *N*-acetyl-L-glutamyl-L-alanine

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Received 29 May 2007; accepted 25 June 2007
Available online 4 July 2007

Abstract—Fluoroolefin peptidomimetics of the dipeptide *N*-acetyl-Glu-Ala were synthesized via an Evans asymmetric aldol reaction and an Overman rearrangement. This dipeptide is the N-terminal of an octapeptide implicated in the signal transduction via PKCε.

The octapeptide EAVSLKPT (Glu-Ala-Val-Ser-Leu-Lys-Pro-Thr) has been shown to inhibit the interaction between protein kinase C-ε (PKCε) and the receptor for activated C kinase 2 (RACK2).[1] PKCε is implicated, in particular, in the transduction of signals involved in cellular proliferation, differentiation, and apoptosis.[2,3] RACK2 is a receptor for the activated form of protein kinase Cε, which localizes PKCε at the appropriate membrane. This localization is essential for the efficient signal transduction.[4,5] RACK2, a homolog of the G protein β subunit, recognizes the amino acid sequence EAVSLKPT within the regulatory domain of PKCε.[3] Physiologically stable inhibitors of the PKCε-RACK2 interaction would provide useful pharmacological tools for a better understanding of the role of PKCε localization in biologic processes.

We thus sought to synthesize an N-terminal peptidomimetic of EAVSLKPT. Many classes of peptidomimetics have been synthesized in recent years, including alkenes, dihydroxyethylenes, hydroxyethylamines, cyclopropanes, and others.[6–10] We report the synthesis of a fluoroolefin peptidomimetic of the dipeptide Glu-Ala. Fluoroolefins are non-hydrolyzable isosteres which replicate particularly well the steric and electronic features of the amide bond (Fig. 1).

Several syntheses of fluoroalkene dipeptide isosteres have been reported in the literature,[11–13] however none

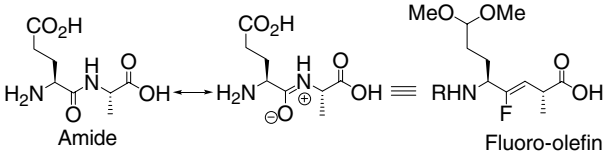

Figure 1. Fluoroolefin isostere of the Glu-Ala amide bond.

describe the synthesis of these peptidomimetics with functionalized side-chains. The synthesis of our precursors is based on the work of Waelchli et al. describing the synthesis of fluoroolefin dipeptide mimics for the synthesis of analogs of the parathyroid hormone.[14] The synthesis is based on an Evans asymmetric aldol reaction followed by an Overman rearrangement. Our peptidomimetic precursor carries a protected aldehyde as a synthetic equivalent of the side-chain acid, which can be oxidized and protected prior to solid phase peptide coupling. Alternatively, it may be more efficient to couple the protected aldehyde and oxidize it after acid-catalyzed deprotection of the full peptidomimetic, and thus avoid additional carboxyl group protection–deprotection steps.

The α-fluoro unsaturated aldehyde was synthesized in four steps from the ester precursor **1** described by Thenappan et al. (Scheme 1).[15] Ethyl (*E*)-2-fluoro-6-hydroxyhex-2-enoate was protected in the presence of *tert*-butyldiphenylsilyl chloride and imidazole in DMF. The ester was treated with $LiAlH_4$ in THF, followed by Collins oxidation. This reagent was chosen for its

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doi:10.1016/j.tetlet.2007.06.154

ability to induce isomerization of the fluoroalkene double bond.[16] The coupling constant of the ethylene proton with fluorine ($J = 32.4$ Hz) confirmed the Z stereochemistry of the alkene.

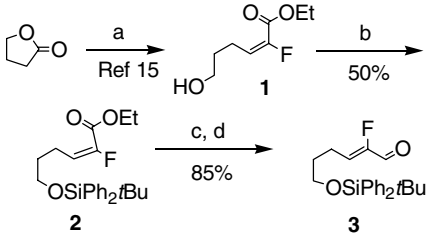
The subsequent step is the formation of two stereocenters via an Evans asymmetric aldol reaction (Scheme 2). Best results were obtained in our case with 2.5 equiv of oxazolidinone, 2.5 equiv of dibutylboryl trifluoromethanesulfonate, 3 equiv of diisopropylethylamine and 1 equiv of aldehyde **3**. The 2(*R*), 3(*R*) stereochemistry of **4**[17] was attributed on the basis of Waelchli's precedent and the Evans' model.[18]

The next step is an Overman imidate rearrangement, following Lurain's procedure (Scheme 3).[19] Product **4** was treated with DBU and trichloroacetonitrile at −5 °C to obtain the corresponding trichloacetimidate. The latter was submitted to thermal rearrangement in refluxing anhydrous toluene. The structure of trichloroacetamide **5**[20] was confirmed by ^{1}H NMR, based on the chemical shift of the amide proton at 6.71 ppm and the coupling pattern (dd, $J = 37.0$, 9.0 Hz) of the signal of the ethylene proton at 5.23 ppm.

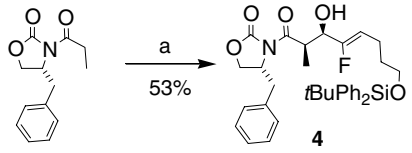
In our first approach, the glutamate side-chain was maintained as a silyl-protected alcohol during the formation of the peptide bond. The hydrolysis of **5** in the presence of EtMgBr in MeOH at 0 °C afforded methyl ester **6**.[21] The ester was hydrolyzed with LiOH and H_2O_2 in THF/H_2O (3:1) to give acid **7** (Scheme 4).

The acid was then coupled to valine benzyl ester with EDCI and HOBt to provide peptide **8**,[22] although the yield remained low (26%) (Scheme 5). After deprotection of the silyl group with aq HF in acetonitrile, alcohol **9** was oxidized with the Dess–Martin reagent. Aldehyde **10** was protected as the dimethylacetal with trimethyl orthoformate in methanol to give **11**. The acetal can be maintained as a carboxylic acid equivalent during solid phase peptide synthesis, while trichloroacetamide could either serve as a protecting group or be maintained in the peptidomimetic. Unfortunately, attempts to hydrolyze the benzyl ester of **11** were unsuccessful, due to the instability of the trichloroacetamide to the hydrolysis conditions.

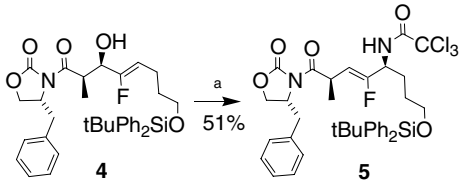
We therefore turned our attention to the N-acetamide in order to mimic the N-terminal amide of the octapeptide within PKCε. Removal of silyl group of **5**, oxidation of the primary alcohol, and protection of the aldehyde gave the desired dimethylacetal **14** (Scheme 6). Reduction of the protected trichloroacetamide **14** under radical conditions, in the presence of tributyltin hydride and azoisobutyronitrile in refluxing toluene, provided the corresponding acetamide **15**. The order of the steps was found to be important, as all attempts to oxidize the side-chain alcohol in the presence of acetamide failed. Hydrolysis of oxazolidinone of **15** followed by



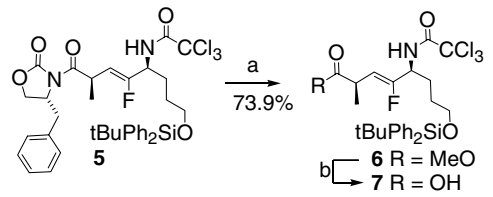
Scheme 1. Reagents and conditions: (a) (i) DIBAL, THF, −78 °C; (ii) triethyl 2-fluoro-2-phosphonoacetate, BuLi, THF, −78 °C; (b) TBDPSCl, imidazole, DMF, rt; (c) $LiAlH_4$, THF, 0 °C; (d) CrO_3, Pyridine, CH_2Cl_2, rt.



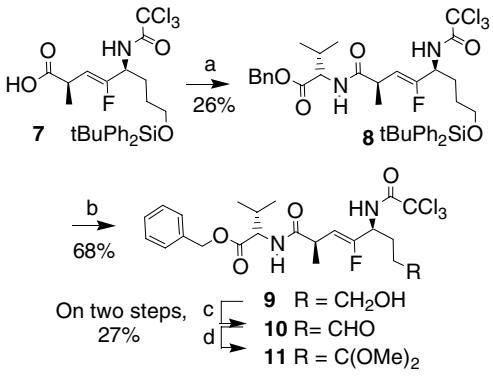
Scheme 2. Reagents and conditions: (a) (i) Bu_2BOTf, DIEA, CH_2Cl_2, −78 °C; (ii) **3**, CH_2Cl_2, −78 °C (2 h) to 0 °C (overnight).



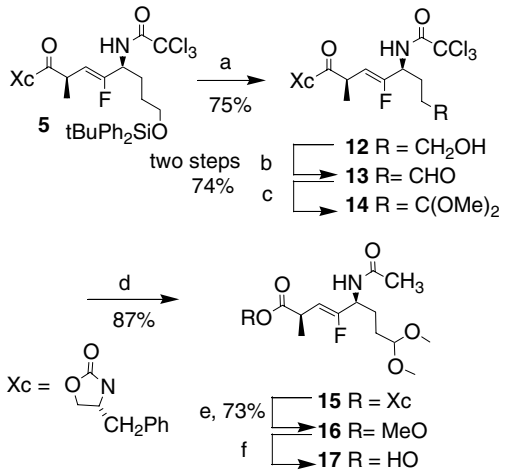
Scheme 3. Reagents and conditions: (a) (i) DBU, CCl_3CN, THF, −5 °C to 20 °C; (ii) toluene, reflux.



Scheme 4. Reagents and conditions: (a) EtMgBr, MeOH, 0 °C to rt; (b) LiOH, H_2O_2, THF: H_2O, 0 °C to rt.



Scheme 5. Reagents and conditions: (a) HCl·H-Val-OBn, DIEA, HOBt, EDCI, DMF; −11 °C (1 h) then 0 °C to rt; (b) aq HF, acetonitrile, rt; (c) Dess–Martin periodinane, CH_2Cl_2, rt; (d) $HC(OMe)_3$, *p*-TSA, MeOH.



Scheme 6. Reagents and conditions: (a) aq HF, acetonitrile, rt; (b) Dess–Martin periodinane, CH_2Cl_2, rt; (c) $HC(OMe)_3$, *p*-TSA, MeOH, rt; (d) Bu_3SnH, AIBN, toluene, reflux; (e) EtMgBr, MeOH, 0 °C to rt; (f) LiOH, H_2O_2, THF–H_2O, 0 °C.

hydrolysis of the resulting ester gave glutamyl–alanine peptidomimetic bearing an N-terminal acetyl group and a dimethylacetal as a protected precursor of the glutamate side-chain acid.

In conclusion, we have synthesized a fluoroalkene peptidomimetic precursor for *N*-acetyl-L-glutamyl-L-alanine. Studies of the solution and solid phase synthesis of the peptide chain are currently underway.[23] The synthesis of other amino acid peptidomimetics from the same precursor is also being investigated.

Acknowledgments

Financial support from the European Union (Contract No. LSHB-CT-2004-503467) is gratefully acknowledged. The authors thank Dr. Damien Ficheux, UMR-CNRS 5086, IBCP-Lyon1, for solid phase peptide synthesis studies.

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17. *Spectral data for* **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68–7.64 (m, 4H), 7.42–7.17 (m, 11H), 4.95 (dt, 1H, $J = 38.8$, 7.3 Hz), 4.68 (dddd, 1H, $J = 9.6$, 6.4, 3.5, 3.4 Hz), 4.55–4.48 (m, 1H), 4.26–4.14 (m, 2H), 3.97 (dq, 1H, $J = 3.5$, 7.1 Hz), 3.69 (t, 2H, $J = 6.3$ Hz), 3.23 (dd, 1H, $J = 13.3$, 3.3 Hz), 2.79 (dd, 1H, $J = 13.3$, 9.2 Hz), 2.29–2.15 (m, 2H), 1.69–1.57 (m, 2H), 1.27 (d, 3H, $J = 7.1$ Hz), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.8, 156.5 ($J_{CF} = 252$ Hz), 152.7, 135.6, 135.0, 134.1, 134.0, 129.6, 129.5, 129.1, 127.7, 127.6, 106.7 ($J_{CF} = 12$ Hz), 69.9 ($J_{CF} = 34$ Hz), 66.3, 63.3, 55.1, 40.3, 37.9, 32.3, 26.9, 19.8, 19.7, 11.0; ^{19}F NMR (188 MHz, $CDCl_3$): δ −124 (dd, $J = 38.2$, 7.3 Hz); HRMS-ESI *m/z*: [MH^+] calcd for $C_{35}H_{43}FNO_5Si$ 604.2895; found, 604.2892; MS (FAB, NBA): 154 (100%), 199 (40%), 604 ([MH+], 16%); $[\alpha]_D^{25}$ −12.55 (*c* 0.239, $CHCl_3$).
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20. *Spectral data for* **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.62 (m, 4H), 7.43–7.17 (m, 11H), 6.71 (d, 1H, $J = 8.6$ Hz), 5.23 (dd, 1H, $J = 37.0$, 9.0 Hz), 4.73 (dq, 1H, $J = 9.0$, 6.9 Hz), 4.63 (dddd, 1H, $J = 9.4$, 6.8, 3.5, 3.3 Hz), 4.47 (dddd, 1H, $J = 20.0$, 8.6, 7.2, 7.2 Hz), 4.25–4.11 (m, 2H), 3.69 (t, 2H, $J = 6.0$ Hz), 3.24 (dd, 1H, $J = 13.3$, 3.3 Hz), 2.78 (dd, 1H, $J = 13.3$, 9.4 Hz), 1.98–1.72 (m, 2H), 1.59–1.49 (m, 2H), 1.35 (d, 3H, $J = 6.9$ Hz), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.0, 174.1, 161.2, 157.6 ($J_{CF} = 260.1$ Hz), 152.8, 135.7, 135.2, 133.8, 129.8, 129.6, 129.1, 127.8, 127.6, 107.6 ($J_{CF} = 11.5$ Hz), 92.4, 66.2, 62.9, 55.3, 52.6 ($J_{CF} =$ 27.9 Hz), 37.9, 34.2, 28.6, 28.3, 26.9, 19.2, 18.5; ^{19}F NMR (188 MHz, $CDCl_3$): δ −121.3 ($J = 37.0$, 20 Hz); HRMS-ESI *m/z*: [MH^+] calcd for $C_{37}H_{43}Cl_3FN_2O_5Si$ 747.1991; found, 747.1990; MS (FAB, NBA) *m/z*: 154 (100%), 137 (78%), 747 ([MH^+], 1%); $[\alpha]_D^{25}$ +11.70 (*c* 0.258, $CHCl_3$).
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22. *Spectral data for* **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65–7.62 (m, 4H), 7.42–7.34 (m, 11H), 6.71 (d, 1H, $J = 8.4$ Hz), 6.05 (d, 1H, $J = 8.8$ Hz), 5.20 (d, 1H, $J =$ 12.2 Hz), 5.10 (d, 1H, $J = 12.2$ Hz), 5.01 (dd, 1H, $J = 36.5$, 9.2 Hz), 4.57 (dd, 1H, $J = 4.7$, 8.8 Hz), 4.55–4.39 (m, 1H), 3.68 (t, 2H, $J = 5.9$ Hz), 3.42 (dq, 1H, $J = 9.2$, 7.0 Hz), 2.15 (qqd, 1H, $J = 7.0$, 6.8, 4.7 Hz), 1.90 (ddt, 1H, $J = 15$, 7.5, 7.5 Hz), 1.82 (ddt, 1H, $J = 15$, 7.5, 7.5 Hz), 1.67–1.55 (m, 2H), 1.26 (d, 3H, $J = 7.0$ Hz), 1.04 (s, 9H), 0.88 (d, 3H, $J = 6.8$ Hz), 0.81 (d, 3H, $J = 7.0$ Hz);

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 171.8, 161.3, 156.7 ($J_{CF} = 259$ Hz), 135.6, 135.3, 133.7, 133.6, 129.8, 128.7, 128.6, 128.4, 127.7, 108.8 ($J_{CF} = 13$ Hz), 92.4, 67.1, 62.8, 56.9, 52.4 ($J_{CF} = 28$ Hz), 36.5, 31.4, 28.6, 28.3, 26.9, 19.2, 19.0, 17.6; ^{19}F NMR (188 MHz, $CDCl_3$): δ −120.6 ($J = 36.5$, 18.1 Hz); HRMS-ESI *m/z*: [MH^+] calcd for $C_{39}H_{49}FCl_3N_2O_5Si$ 777.2460; found, 777.2462; MS (ESI) *m/z*: 799.2 ([$M+Na]^+$, 85%), 779 ([$M+H]^+$, 100%); $[\alpha]_D^{25}$ −29.7 (*c* 1, CH_2Cl_2).

23. Preliminary attempts to couple the *N*-acetyl peptidomimetic under either solution or solid phase conditions have been unsuccessful.