Bioorganic & Medicinal Chemistry Letters, Vol.2, No.6, pp. 571-574, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

HBTU: A MILD ACTIVATING AGENT OF MURAMIC ACID

Solange Adam
F. Hoffmann-La Roche AG. CH-4002 Basel (Switzerland)

(*Received* 26 *March* 1992)

Summary: O-Benzotriazolyl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) effects the smooth coupling of 1, a derivative of muramic acid, with alcohols, peptides and amines in high yields.

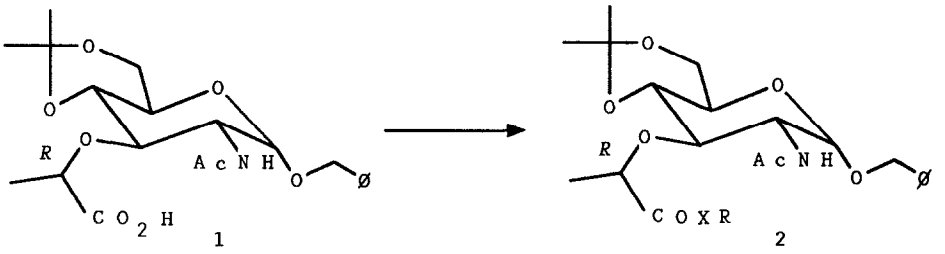
In connection with our work done in the area of immunomodulation and adjuvants for vaccines we were in need of an efficient, smooth and high yielding method for the coupling of the costly or multistep available muramic acid derivative[1] 1 with different partners: peptides, amines and alcools.

At the outset of our work we prepared the activated ester 2a via the well established N-hydroxysuccinimide/dicyclohexylcarbodiimide[2] method. Unfortunately the ester 2a was accompanied by the formation of the well known side product N-acylurea which amounted to 50% yield, therefore we envisaged to tackle this problem by using **HBTU** which is already successfully used for the coupling of amino acids[3]. We found[4] that **HBTU** is also a mild activating reagent of the glucoacid 1 for its coupling with peptides. The coupling is caracterised by its mildness and the high yields obtained. This result prompted us to evaluate the potential of **HBTU** for the coupling of the muramic acid derivative 1 with alcohols, amines and more elaborated peptides than already described[4]. As to our knowledge there has been no other publication[4], about its use in sugar chemistry, in this paper we report the results obtained with the substrates shown on the Scheme.

For further elaboration we needed a temporarily[5] protecting group

for the acid 1 which could be easily cleaved and could also survive the hydrogenolysis of the anomeric protecting benzyl group[6]. To this end, the 2-(trimethylsilyl)ethyl ester 2b was synthesised according to the following protocol:

Scheme



2a; XR: $-O-N(CO-CH_2)_2$

2b; XR: $-OCH_2CH_2Si(CH_3)_3$

2c; XR: $-HN(CH_2)_{11}CH_3$

2d; XR: -*L*-Ala-*D*-isoGlu-Nϵ(CBz)-*L*-Lys-*D*-Ala-*D*-Ala-OBz

Under argon, a stirred solution of 1 (2.1 g, 5 mmol) in 20 ml CH_2Cl_2 and 20 ml acetonitrile was treated rapidly at rt by the sequential addition of 4-dimethylaminopyridine (DMAP) (0.61 g, 5 mmol), HBTU (1.89 g, 5 mmol) and 2-(trimethylsilyl)ethanol (0.65 g, 5.5 mmol). The clear reaction mixture was let stir overnight. The solvent was evaporated and the residue was purified by flash chromatography (n-hexane-CH_3CO_2Et, silica gel) to give 2b as a colourless oil in 80% yiel.

2b: $[\alpha]^{20}$ = + 117.6° (c=1, $CHCl_3$). ^1H NMR ($CDCl_3$, 250 MHz): δ 7.5 (d, NH); ~ 7.19-7.35 (m, aromatics); 5.335 (d, J = 4 Hz, H_1); ~ 4.64 and ~ 4.43 (2xd, J_{gem}= 12 Hz, O-CH_2Ph); 4.375 (q, J = 6 Hz, C*H*-CH_3); 4.2-4.14 (m, -CO_2CH_2-); ~ 3.82-3.52 (m, H_2,H_3,H_4,H_5, H_6); 1.97 (s, CH_3CO-NH); 1.44 and 1.33 (2xs, 2xCH_3); ~ 1.305 (d, CH_3-CH-); 0.99-0.92 (m, -CH_2-Si); ~ 0.0 [s, $(CH_3)_3$Si-] ppm.

Typical procedure for the condensation with amines:
the amide 2c was synthesised according the following procedure: under argon, a stirred suspension of 1 (4.2 g, 10 mmol) in 50 ml acetonitrile was treated at rt with NEt_3 (1.6 ml, 11.5 mmol) and

DMAP (0.25 g, 2 mmol). The resulting solution was then treated with HBTU (3.8 g, 10 mmol). After 7h, the solvent was evaporated and the residue was partitioned between CH_3CO_2Et and water. The organic phase was separated, washed successively with cold 1N HCl, water, dilute cold $NaHCO_3$aq. and water, dried, filtered and evaporated. Purification of the oily residue by flash chromatography (CH_3CO_2Et, silica gel) furnished 2c as a dick colourless oil.

2c: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.3 (d, NH); ~ 7.43-7.24 (m, NH, aromatics); 4.895 (d, J = 3.5 Hz, H_1); 4.66 and 4.46 (2xd, J_{gem}= 12 Hz, O-CH_2Ph); 4.08 (q, J = 7 Hz, -C*H*-CH_3) ppm.

The pentapeptide-part of 2d was synthesised starting from D-Ala-OBz by the sequential addition of the adequately protected amino acids or peptide (L-Ala-D-isoGlu[7]) using the methodology described in this paper. The final N-Boc-protected pentapeptide was further processed for the coupling with 1 as described in the following two-step procedure:

Thus, N-Boc-L-Ala-D-isoGlu-Nε(CBz)-Lys-D-Ala-D-Ala-OBz (0.89 g, 1.1 mmol) was added under stirring and cooling at -10°C (ice-MeOH bath) to 3.2 ml trifluoroacetic acid. After 30 min the acid was rapidly removed under reduced pressure at 20°C. Benzene (2 ml) was added and rapidly evaporated. This treatment was repeated twice to remove most of the acid. The residue was dissolved in CH_2Cl_2 (10 ml) and treated with NEt_3 (0.22 ml, 1.6 mmol). This solution was added under stirring at rt to a solution of 1 (0.47 g, 1.1 mmol), DMAP (0.135 g, 1.1 mmol), HBTU (0.42 g, 1.1 mmol) and NEt_3 (0.44 ml, 3.1 mmol) in 100 ml CH_2Cl_2 and 20 ml acetonitrile. After 3h the solvent was removed under reduced pressure and to the residue was added ice-water. The crystalline suspension was stirred for 5 min, then filtered. The colourless crystals were thoroughly washed with water, dissolved in MeOH and benzene. The clear solution was concentrated under reduce pressure whereupon 2d crystallised out furnishing 0.98 g (0.88 mmol, 80% yield) of colourless crystals .

2d: ^{1}H NMR ($DMSOd_6$, 400 MHz) δ 8.28, 8.17, 8.16,8.11, 8.01 and 7.45 (6xd, 6 NH); ~ 7.4-7.26 (H-Ph, -CO-NH_2, NH); 7.23 (2xd, N*H*-CH_2-); 7.07 (broad s, -CO-NH_2); 5.10 (ABq, O-CH_2Ph); 4.99 (~ s, O-CH_2Ph); 4.81 (d, J = 3.5 Hz, H_1); ~ 4.67 and 4.47 (2xd, J_{gem}= 13 Hz, anomeric-OCH_2Ph); ~ 4.35-4.25 (m, 3H, Ala-C*H*-N); ~

4.21-4.11 (m, 2H,isoGlu-C*H*-N, α-Lys-C*H*-N); 4.09 (q, J = 6.5 Hz, lactyl -C*H*-CH_3); ~ 3.79-3.66 (m, 3H); ~ 3.58-3.50 (m, 2H); 2.5 (m, HN-CH_2-); 2.11 (m, 2H, -OC-CH_2-); 1.9 (m, 1H, OC-CH_2-CH_2-); 1.80 (s, CH_3CO-N); 1.7 (m, 1H, OC-CH_2-CH_2-); ~ 1.6-1.1 (m, 3x -CH_2-, 2xCH_3, 4x CH_3-C*H*) ppm.

Acknowledgement: our thanks are due to our colleagues of Hoffmann-La Roche Central Research for spectral data and elemental analyses.

REFERENCES AND NOTES

1. A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso and I. Azuma, *Agric. Biol. Chem.* 42, **1978**, 2187-2189.
2. J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.* 77, **1955**, 1067-1068.
3. V. Dourtoglou, J. C. Ziegler and B. Gross, *Tetrahedron Lett.* 15, **1978**, 1269-1272.
4. S. Adam and F. Kaufmann, *Chem. Phys. Lipids*, 59, **1991**, 255-261.
5. P. Sieber, *Helv. Chim. Acta*, 60, **1977**, 2711-2716.
6. The hydrogenolysis of the benzyl group of 2b proved to be a smooth and a quantitative one.
7. available from Bachem (Switzerland).