Predictable half-life extension demonstrated through the peptide TRI-1144

Eric L. Schneider, Gary W. Ashley, Louise Robinson, Ralph Reid, Lieve Dillon, Bart Stoops, Nigel Austin, Bruce Malcolm*, Daniel V. Santi

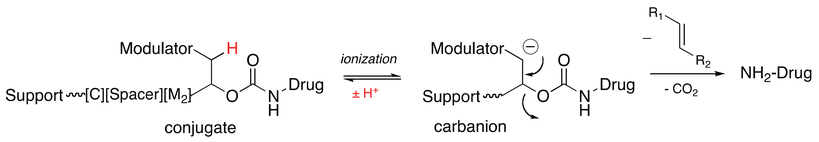
ProLynx LLC, Hayward, CA and Janssen Research and Development, Beerse, Belgium

Eric@ProLynxLLC.com

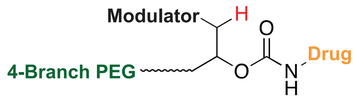
INTRODUCTION

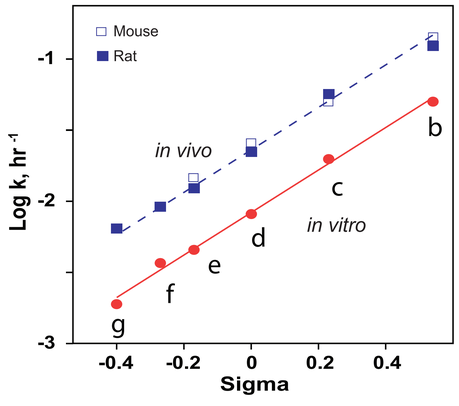
ProLynx recently reported a new format for releasable macromolecule-drug conjugates that does not require enzyme hydrolysis, is highly tunable, and gives predictable cleavage rates both *in vitro* and *in vivo* (Santi DV et al. (2012) PNAS 109:6211-6216). The technology uses novel linkers that undergo β-elimination reactions at pre-programmed rates to release drugs from macromolecular conjugates, e.g. polyethylene glycol (PEG). These linkers were shown to provide predictable, tunable release rates over a range spanning hours to months at physiological pH and were used to increase the in vivo clearance half-life of exenatide by > 50-fold in rat (from 30 min to 28 hours). In collaboration with Janssen Research and Development, application of the technology to the 38 amino acid fusion inhibitor reference peptide TRI-1144 (preciously intended for once-a-day treament of HIV) demonstrated the technology's predictability. From the *in vitro* release kinetics and drug pharmcokinetics we were able to successfully identify a linker and dosage on the first pass that would provide the desired minimal concentration with once-a-week dosing. By conjugation of the peptide to 40 kDa PEG via the appropriate ProLynx linker, the free peptide clearance half-life was increased 8-fold in rats, from 4 hrs to 34 hrs, keeping the free peptide concentration in plasma above 4 nM over 7 days with an initial injection of 3.6 mg/kg of peptide .

β-ELIMINATION CHEMISTRY UTILIZED BY PROLYNX



A modulator controls the ionization rate and therefore the rate of elimination and release of drug. *In vitro* and *in vivo* kinetics studies demonstrate that a wide range of release half-lives can be generated by adjusting the electronics of the modulator and that there is a correlation between the electronics of the modulator and the measured release rates. This correlation indicates the ability to predictably design a linker with any desired half-life.





PREDICTING DRUG HALF-LIFE EXTENSION IN VIVO

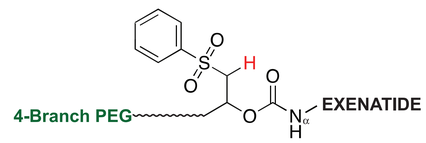
Knowledge of the *in vitro* release rates of the linkers combined with known *in vivo* pharmacokinetics of the drug and the macrimolecular carrier, predictions of the *in vivo* half-life extension and drug concentration can be made using the following equations.

1) $k_{drug} = k_1 + k_3$

2) $[\text{Drug}]_{\text{rel, ss}} = k_1/k_2[\text{PEG-Drug}][V_{Conj}/V_{Drug}]$

where k_{Drug} is the released drug clearance rate constant, k_1 is the linker release rate constant, k_2 is the drug clearance rate constant and k_3 is the PEG clearance rate.

APPLICATION TO HALF-LIFE EXTENSION OF EXENATIDE



Given the known values for Exenatide and the PEG-Exenatide conjugate, the following prediction for Exenatide was made:

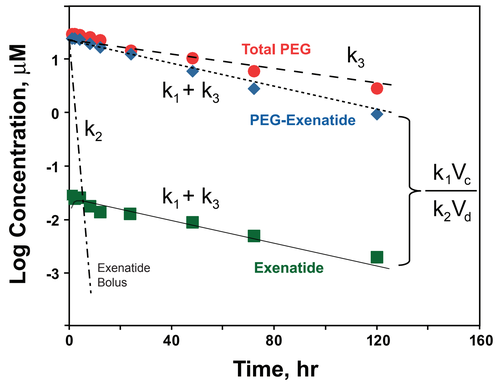
Known exenatide values

linker $t_{1/2}$	133 hr
Exenatide $t_{1/2}$	0.5 hr
PEG $t_{1/2}$	44 hr
V_{PEG}	0.035 L/kg
$V_{Exenatide}$	0.4 L/kg

Predicted in vivo results

linker $t_{1/2}$	45 hr
Exenatide $t_{1/2}$	22 hr

EXPERIMENTAL RESULTS CONFIRM PREDICTION



Calculated $t_{1/2}$ values:

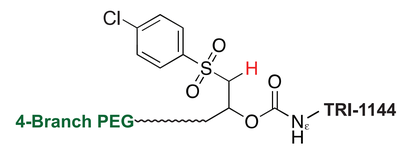
PEG: 45 hours

Exenatide: 28 hours

In vivo cleavage: 78 hours

In vivo rat PK studies show the expected increase in half-life of exenatide (to 28 hours).

APPLICATION OF HALF-LIFE EXTENSION TO TRI-1144



PREDICTION OF TRI-1144 BEHAVIOR IN VIVO

Known TRI-1144 values

linker $t_{1/2}$	690 hr
TRI-1144 $t_{1/2}$	4 hr
PEG $t_{1/2}$	44 hr
V_{PEG}	0.035 L/kg
$V_{TRI-1144}$	0.15 L/kg

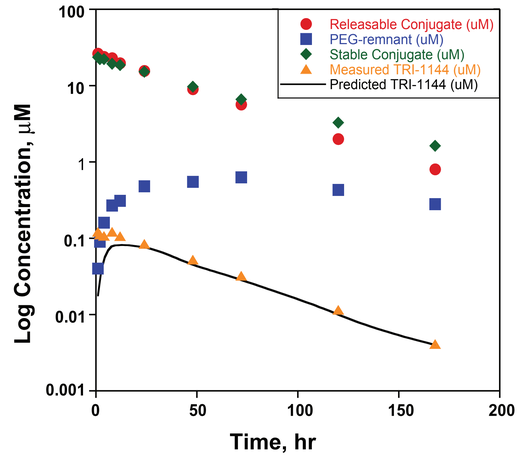
Predicted in vivo results

linker $t_{1/2}$	230 hr
TRI-1144 $t_{1/2}$	37 hr
$C_{7\ days}$	4.7 nM
C_{max}	75 nM

Predicted concentrations based on 800 uM solution doesd at 1 ml/kg.

RAT PK RESULTS FOR TRI-1144 CONJUGATE MATCH PREDICTED BEHAVIOR

As with the Exenatide, the PK results for TRI-1144 match very closely with the prediction.



Measured $t_{1/2}$ values:

PEG: 44 hours

Free TRI-1144: 34 hours

In vivo cleavage: 150 hours

$C_{7\ days}$: 4.2 nM

C_{max}: 120 nM

CONCLUSION

The ProLynx releaseable linker technology utilizes novel β-elimination chemistry that has been experimentally demonstrated to be highly predictable both *in vitro* and *in vivo*, allowing for rapid and reliable selection of any desired half-life. *In vitro* and *in vivo* studies on releasable model conjugates, including the peptide exenatide, have given us the ability to accurately predict the behavior of additional peptides *in vivo*. A collaboration with Janssen Research and Development demonstrated this predictability by extending the clearance half-life of TRI-1144 8-fold and maintaining a C_{min} of 4.2 nM over one week.